5-HYDROXYTRYPTAMINE NEUROTRANSMISSION IN A BULBO-SPINAL PATHWAY

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SUMMARY

This study has investigated the effect of electrical stimulation of a 5-HT-containing pathway on the excitability of lumbar motoneurones.

The modification of motoneurone excitability has been assessed by i) recording intracellularly from motoneurones, ii) recording extracellular motoneurone field potentials and iii) monitoring spinal reflexes. Identification of the involvement of 5-HT in the effect has been demonstrated by pharmacologically blocking the response with three different 5-HT antagonists and by facilitating the effect by increasing neuronal 5-HT levels.

Iontophoretic application of agents close to lumbar motoneurones has indicated that activity in the raphe-spinal pathway is capable of modifying motoneurone excitability, the effect possibly being mediated via 5-HT close to lumbar motoneurones.

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INTRODUCTION

I N T R O D U C T I O N

It is intended to briefly present the facts which form the basis of our present understanding of the role of 5-HT (5-hydroxytryptamine or serotonin) systems within the mammalian central nervous system (CNS).

The data concerning the biochemistry of 5-HT is presented only where it has direct and obvious relevance to the physiological and pharmacological problems which have been examined. Restriction of this account to the central actions of 5-HT, primarily at spinal levels, is necessary in view of the grossly different role this substance seems to have in the periphery. However, it must be remembered that until 1935 interest in 5-HT, based upon its distribution within the gastro-intestinal tract and blood platelets, was concentrated upon its possible involvement in the regulation and coagulation of the cardiovascular circulation. Rapport, Green and Page (1948) purified and characterised a vasoconstrictor substance which they named serotonin. This later proved to be identical with 'enteramine' which had been investigated much earlier (Erspamer and Vialli, 1937).

Twarog and Page (1953) first reported the presence of 5-HT in brain tissue. Further studies indicated an uneven distribution of 5-HT within the CNS (Amin, Crawford and Gaddum, 1953; Vogt, 1954; and Bogdanski, Weissbach and Udenfriend, 1958). The functional implications of this observation, as a generalisation were profound; it had long been accepted that transmitter substances are found in those neurones from which they are released. Thus the disparate distribution of 5-HT immediately

suggested the indole might be involved in synaptic transmission.

A clarification of some of the aspects of the metabolic pathways of indole compounds at this point, will probably assist in the understanding of some of the following reported work. The metabolic pathways which have received most attention are illustrated in Fig. 1. The amino acid tryptophan is the dietary precursor of 5-hydroxyindole compounds (Udenfriend, Titus, Weissbach and Petersson, 1961). These authors concluded that 5-hydroxytryptophan (5-HTP) was the first hydroxylated product, the conversion is catalysed by the enzyme tryptophan hydroxylase. This step is probably rate-limiting in the anabolism of 5-HT.

The peripheral administration of 5-HT does not enter the central nervous system (except in large doses in mice, Udenfriend, Weissbach and Bogdanski, 1957), whereas the peripheral administration of 5-HTP (Udenfriend, Bogdanski and Weissbach, 1956) or L-tryptophan (Hess and Doepfner, 1961) increases the neuronal 5-HT level. 5-HTP is decarboxylated by 5-HTP decarboxylase, an enzyme which has a distribution similar to that of 5-HT. The chief product of 5-HT degradation is 5-hydroxy-3yl-indole acetic acid (5-HIAA). The process is one of oxidative deamination by monoamine oxidase - an enzyme which is located within presynaptic mitochondria. Monoamine oxidase is unlikely to be entirely responsible for terminating any synaptic activity 5-HT may have, this is almost certainly effected via a re-uptake mechanism, the indole being taken up into the presynaptic terminal.

Many of the techniques used to investigate the physiological

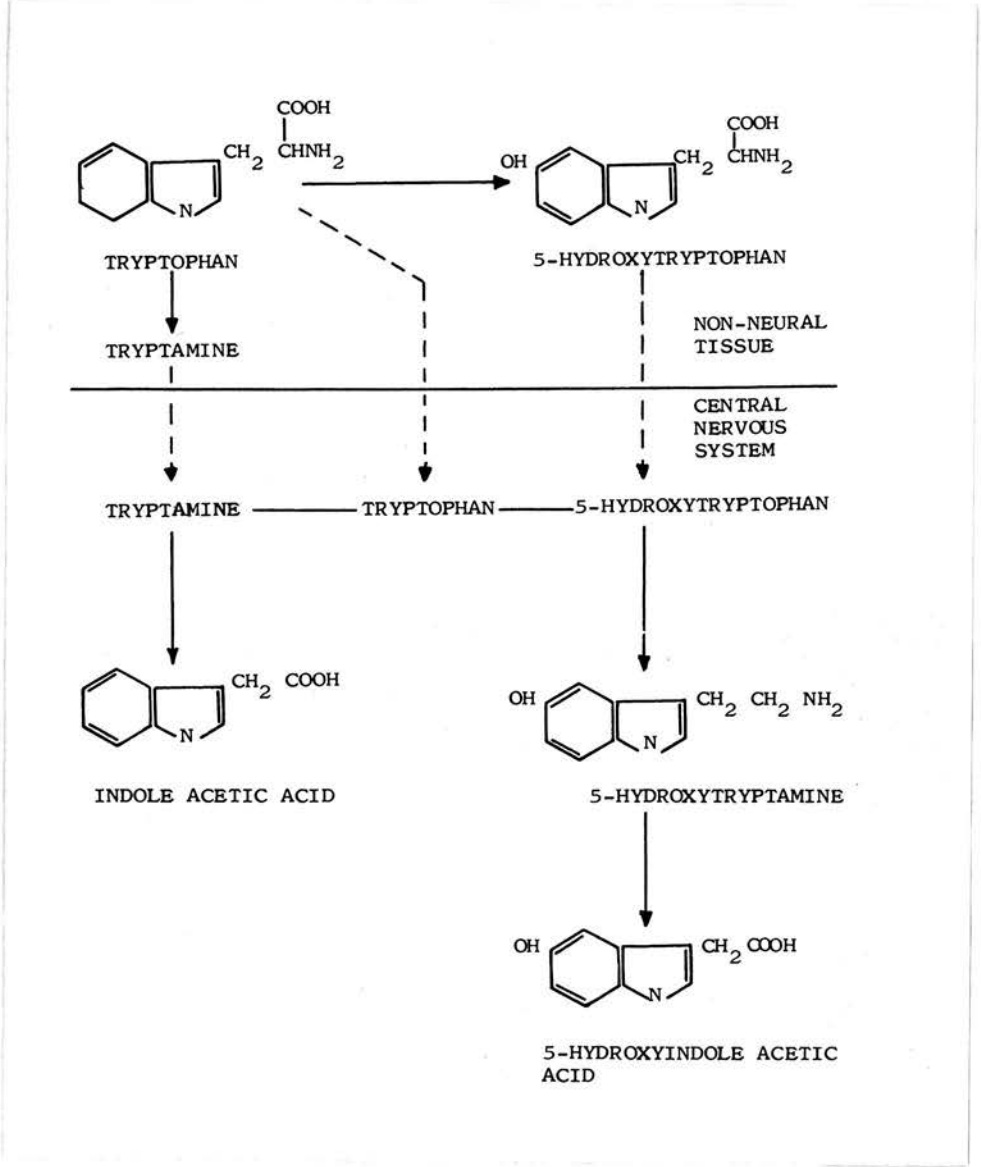

FIGURE 1

Metabolism of tryptophan and related compounds in relation to the central nervous system. (adapted from D.F. Sharman 1965).

and pharmacological action of 5-HT within the central nervous system have involved disrupting the metabolism of 5-HT. Thus precursor loading experiments have involved the administration of either tryptophan or 5-HTP, resulting in an increase of 5-HT levels (Bogdanski et al., 1958). On the other hand, drugs which block the enzymes responsible for the anabolism of 5-HT will obviously reduce the rate of 5-HT synthesis. Secondary effects of many of these pharmacological tools unfortunately restrict their usefulness. For example, 5-HTP was at one time frequently used as the major precursor for 5-HT, however recent work suggests that a large proportion of the 5-HT formed from 5-HTP is not localised interneuronally, wherease L-tryptophan loading increases levels of presynaptic 5-HT (Carlsson, 1960; Fuxe, Butcher and Engel, 1971). Similarly para-chlorophenylalanine (PCPA) has long been regarded as a 5-HT-depleting agent - Crossland (1970) has recently stated that "p-chlorophenylalanine depletes rat and mouse brain of their serotonin without influencing the synthesis of noradrenaline". This comment is in direct conflict with biochemical evidence implicating PCPA as an inhibitor of phenylalanine hydroxylase and tyrosine hydroxylase (McGeer, Peters and McGeer, 1968), and an agent in reducing brain noradrenaline levels (Lipton, Gordon, Guroff and Udenfriend, 1967; Welch and Welch, 1968). Further examples of the misuse of pharmacological agents will be discussed at the relevant point.

The above techniques, amongst others have been used to study the possible role of 5-HT in bodily functions. The recent recognition of the limitations of the techniques

employed has in some cases meant that a reappraisal of the data is required.

These and other experimental techniques have suggested that 5-HT may be involved in several bodily functions. Pscheidt .et al. (1964), noting that 5-HT is distributed within the phylogenetically older structures of the brain has suggested that the indole may be partly responsible for the activities of these structures. Previously Brodie and Shaw (1957) had suggested that serotonin may mediate the trophotropic system outlined by Hess. Serotonin would, it was postulated, be responsible for the integration of the 'recuperative type' behavioural patterns i.e. sleep and hibernation. In such a role serotonin would oppose a noradrenergic influence in the form of the ergotropic system. The latter system Hess proposed to be responsible for an increased level of arousal and generally increased sympathetic activity.

The concept of 5-HT being involved in sleep mechanisms has been partially substantiated by recent work. The parent somata of all 5-HT-containing neurones in the central nervous system correspond to the raphe nuclei situated at the border of the midbrain and hindbrain (Dahlstrom and Fuxe, 1965). Work by Jouvet (1962), Pujol, Buguet, Froment, Jones and Jouvet (1970) and Renault (1967) has suggested that the integrity of the nuclei raphe is essential for normal sleep patterns. Complete destruction of the raphe nuclei in cat by electrolytic coagulation produces an almost totally insomniac animal (Pujol et al., 1970).

A similar state can be induced by pharmacologically

depleting the nervous system of 5-HT, small doses of 5-HT-precursors will reintroduce normal sleep patterns. Little however is known regarding the mechanism of the action of 5-HT and there is sufficient evidence to suggest that activity in at least one other monoamine system (noradrenaline) is essential for normal sleep processes.

5-hydroxytryptamine has also been implicated in the behavioural manifestation of sexual activity. Recent work (Shillito, 1969) has suggested that pharmacologically lowering brain 5-HT levels may correspond to aberrant sexual activity, however the relationship between 5-HT levels and the behavioural activity is far from clear.

A more firmly established role for the 5-HT system is that of thermoregulation. Experimentally raising the body temperature of a rat has been shown to accelerate 5-HT metabolism, (Weiss and Aghajanian, 1970). Measurement of the primary metabolite of 5-HT, 5-HIAA, indicated that a rise in body temperature is accompanied by a rise in forebrain 5-HIAA levels. These authors demonstrated the interdependence between the raphe nuclei and the forebrain 5-HIAA levels by lesioning the midbrain in the region of the raphe nuclei. Subsequent increases in body temperature were not accompanied by changes in 5-HIAA levels. Confirmation that activity of raphe nuclei neurones is associated with changes in body temperature was elegantly demonstrated by the same authors. Microelectrode recordings from single raphe units indicated that as body temperature rises, there is an increase in firing rate. Non-raphe units in the neighbouring region did not display this

response.

During the last fifteen years there has been an increasing awareness of a possible link between the activity of monoamine systems within the CNS and the affective disorders. This name is given to a group of mental disorders in which the basic upset is one of the emotions (affect). An individual experiencing this type of illness may vary from a state of extreme elation to one of profound depression.

One of the earliest explicit formulations of a theory linking brain monoamine levels with the affective disorders was initiated by Jacobsen (1964) and expanded by Schildkraut (1965). The theory is based upon the hypothesis that reduction of activity at synapses where noradrenaline acts as a transmitter may be responsible for some of the symptoms of depression. The evidence for the theory is centred upon the action of drugs such as reserpine which deplete synaptic terminals of noradrenaline. Holzbauer and Vogt (1956) and Carlsson, Rosengren, Bertler and Nilsson, (1957) were the first to show that reserpine causes an almost complete depletion of brain catecholamine stores.

There have been many reports concerned with the mode of action of reserpine, and two different theories have become apparent. The Scandinavian workers, whilst agreeing that reserpine might interfere with serotonin transmission, suggest that the primary target for reserpine is the catecholamine system. The emphasis on this system derives from an observation by Carlsson (1961) who showed that whilst a precursor of noradrenaline (dopa) could restore normal behaviour in a

reserpinised animal, the precursor of 5-HT (5-HTP) induced tremors, convulsions and abnormal postures.

The alternative hypothesis states that the sedative action of reserpine is mainly mediated through an action on serotonin rather than catecholamines. Similarly there is little agreement concerning the exact mechanism of action of reserpine, however there does appear to be a reserpine-stable pool and a reserpine-labile pool of amine in the brain. Probably reserpine has its action on an intracellularly located labile fraction, possibly by blocking storage in the storage vesicles or granules. After 5 mg/kg reserpine, the 5-HT and noradrenaline levels in the brain of rabbit are profoundly reduced (Dahlstrom and Fuxe, 1965). In animals, reserpine produces a syndrome of sedation which has been compared to depression in man (Sulser 1964). Increasing the efficacy of noradrenergic transmission by either blocking the activity of the main degradative enzyme - monoamine oxidase - or by decreasing the efficiency of the uptake mechanism, may be logically expected to relieve depression.

Evidence that the tricyclic antidepressants may effect their actions via the latter mechanism has been recently provided. Imipramine, desipramine (DMI) and, to a lesser extent, amitryptyline (all in doses of 20 mg/kg) were found to inhibit the accumulation of (H^3)-noradrenaline injected intraventricularly to rats (Glowinski and Axelrod, 1964, 1966). More recently it has been shown that tricyclic antidepressants, especially the tertiary amines like imipramine and amitryptyline, potently inhibit 5-HT uptake into brain slices; secondary amines

such as DMI are less effective (Ross and Renyi, 1967; Carlsson, 1970). Studies involving spinal reflex activity (Meek, Fuxe and Anden, 1970; Clineschmidt, Pierce and Sjoerdstama, 1971) have generally confirmed the effect, however the use of 5-HTP as the serotonin precursor again limits the significance of the observations.

Chlorpromazine is a representative of another group of psychoactive drugs, the tranquillisers. Despite the fact that chlorpromazine has been widely used since 1953, there is relatively little information available concerning the precise mode of action by which it produces its clinical effect. The iontophoretic application of chlorpromazine onto single cells in the cat brain stem reticular formation indicated that the drug blocked the excitatory effects of noradrenaline, but not the inhibitory effects (Bradley, Wolstencroft, Hosli and Avanzino, 1966). More recently, it has been reported that iontophoretically applied chlorpromazine can block both excitatory and depressant responses of dopamine in the putamen (York, 1971). Although 'local anaesthetic' actions of chlorpromazine (Bradley et al., 1966) have to be taken into account when considering the disturbance of monoamine transmission, dopamine receptor blockade may account for the numerous extrapyramidal disturbances, resembling Parkinsonism, which have been reported (Ayd, 1961; Duvoisin, 1968). However the situation is further complicated by the uptake blockade properties exhibited by this drug (Iversen, 1965).

In a recent review, Davies (1970) has emphasized the paucity of direct evidence implicating any single one of the

biogenic amines in behavioural disorders. Many problems arise from the difficulty in assessing the clinical efficacy of therapeutic agents. In addition, diagnostic identification of mania and depression cannot be wholly objective, the periodic nature of the psychosis also makes clinical evaluations difficult. Assuming 5-HT, noradrenaline (NA) and dopamine (DA) are all involved in synaptic transmission, then a therapeutic drug may have its effect through the integrated action on several, rather than one, transmitter, i.e. the balance between transmitter actions may be important.

For several years it was suggested that some forms of schizophrenia were accompanied by low brain levels of 5-HT (Sidman, 1956; Wooley and Shaw, 1954). The investigations of Brengelman, Pare and Sandler, (1958) at first yielded the promising observation that 5-HTP diminishes the psychological effects of LSD. However, 5-HTP failed to alleviate the clinical symptoms of schizophrenics, thus reducing the possibility that a reduction in the efficacy of 5-HT transmission underlies this illness (Roth, 1961). The theory had also been based upon the similarities between some LSD effects and some schizophrenic episodes and the discovery that the contractile action of 5-HT on smooth muscle was antagonised by LSD (Gaddum, 1953; Gaddum and Hameed, 1954).

Few would claim that LSD produces a clinically recognisable schizophrenia, and although LSD 25 is capable of producing a schizophrenic-like state in certain subjects, the circumstances under which the drug is given and the dose administered are important in determining the type of response elicited.

It is important to recognise the unsatisfactory nature of the classification of 'hallucinogenic' or 'psychotomimetic' drugs. These drugs are grouped together on the basis of their ability to alter perception (primarily in the form of producing visual hallucinations) in man. However, there is no satisfactory method for distinguishing accurately between the different psychological states induced by different psychotomimetics. Biochemical studies of the effects of hallucinogens on central monoamine systems have provided some firm evidence concerning the possible effects of such drugs, (see below). However at doses which induce psychological disturbances in man, no corresponding biochemical anomalies are recorded in animals. Tonge and Leonard (1969), for example, obtain significant biochemical changes only following 3 mg/kg LSD ir the rat, a dose 1000 - 3000 times greater than the human hallucinogenic dose.

Despite the evidence implicating 5HT as a mediator of the action of hallucinogenic and psychoactive drugs, and suggesting an involvement in mental illness, little attempt has been made to study the physiology of the monoamine pathways. In some respects this is understandable, the monoamine system generally and the 5-HT pathway in particular, are especially difficult to study using normal electro-physiological techniques. Axons emanating from 5-HT containing soma are fine, unmyelinated fibres, (Dahlstrom and Fuxe, 1964). Such fibres have relatively high firing thresholds. There is thus a distinct danger that neighbouring structures having lower thresholds will also become excited. Engberg, Lundberg and

Ryall, (1967) have emphasised the problems associated with electrically stimulating monoaminergic pathways.

In addition, there is still considerable confusion concerning a possible neurotransmitter role for 5-HT. Many reviewers have discussed the basic requirements which must be demonstrated for any putative synaptic transmitter before it may be regarded as having transmitter action (Eccles 1964, McLennan 1970, Curtis 1961, Hebb 1970, Werman 1966).

As concepts of metabolism, storage release and inactivation of transmitter substances have changed, so have the basic requirements for the identification of a transmitter substance. Werman (1966) in a recent review has outlined the criteria to be fulfilled by a suspected transmitter.

Probably the first criterion to be met in this context is the presence of the putative transmitter substances at the relevant sites. When considering the distribution within the CNS, probably the most significant recent development has been in the field of histochemical fluorescence. Falck (1962) made the important discovery that freeze-dried nervous tissue treated with paraformaldehyde, condensed to form highly fluorescent derivatives. The technique was found to be applicable to freeze-dried tissue sections, allowing the localisation of 5-HT by fluorescence microscopy. The chemistry of the reaction, as described by Dahlstrom and Fuxe (1964) and Falck and Owman (1965), involves the production of a highly fluorescent quinoline. A yellow light is observed when the sections are viewed under ultra-violet light. The monoamines may be identified by their different fluorescence

characteristics. Recent evidence suggests however that the concentration of the amine to some extent determines the wavelength of the emitted light (Dow, Laszlo and Ritchie, 1972).

The technique, although extremely valuable as a research tool, is not easy to perform: the experimental conditions during the condensation stage are critical. The stability of the quinolines is very low and care must be taken to measure the fluorescene within a set period.

Dahlstrom and Fuxe (1965) have slightly modified the fluorescence technique for the investigation of monoamine terminals in spinal cord tissue. This part of the nervous system seems to require a slightly higher water content at the sites of the histochemical reaction. The fluorescence due to 5-HT in the spinal cord is often not very great and may be confused with fluorescence derived from other monoamines. 5-HT terminals are best studied in animals treated with nialamide (a monoamine oxidase inhibitor) and reserpine. Although reserpine normally depletes the nervous system of the monoamines, when administered with nialamide it increases the accumulation of 5-HT. Occasionally nialamide has been administered in conjunction with the immediate 5-HT precursor, 5-HTP. However in the light of present knowledge suggesting that 5-HTP leads to accumulation of 5-HT in terminals which would naturally contain no 5-HT (Pujol, 1971), this procedure seems rather suspect.

A method for removing the catecholamine fluorescence involves the incorporation of a 'false transmitter' into the normal metabolic pathway. For example, administration

of meta-tyrosine causes a marked decrease or complete disappearance of catecholamine terminal fluorescence within two hours, whilst 5-HT fluorescence remains normal (Dahlstrom and Fuxe 1964). An examination of the distribution of monoamine-containing cell bodies and terminals (Dahlstrom and Fuxe 1964; Carlsson, Falck, Fuxe and Hillarp 1964) has indicated that the systems of 5-HT-containing terminals present in the different areas of the brain and spinal cord may be derived from neurone groups in the lower brain stem - these groups have been identified as belonging to the nuclei of the raphe.

The raphe nuclei in cat have been studied in detail using Nissl and silver staining techniques (Taber et al., 1960; Brodal et al., 1960, 1960a). The term raphe means a line, seam or ridge and this aptly describes the location of the nuclei. In cat the nuclei extend from the caudal pole of the medial accessory olive to the rostral mesencephalon. On a cytoarchitectonic basis the raphe complex has been divided into 8 nuclei. However the transition from one group to the next is indistinct. Taber et al., (1960) note the similarity between some of the nucleus of the raphe and the adjacent reticular formation. One nucleus in particular (nucleus raphe magnus) is regarded as being fused with the neighbouring reticular formation (nucleus reticularis gigantocellularis).

The afferent and efferent connections of the various nuclei are not always apparent. Whilst many nuclei project predominantly to one area of the CNS, many of the axons

originating from raphe neurones divide into an ascending and a descending branch (Cajal 1909-1911).

Degeneration studies (Brodal et al,, 1960a) indicate that the nucleus raphe magnus (n.r. magnus) and to a lesser extent n.r. pallidus, are the chief source of spinal fibres. Other nuclei seem to be little involved in the spinal projection system. Ascending fibres emanate from the two most caudal nuclei and the three rostral ones. It is interesting to note that the two nuclei responsible for the spinal outflow also receive the majority of spinal afferent fibres. A summary of Taber's observation is given below (see Table 1).

Caudal

Origin/Termination of Fibres	Ascending	Descending	Cerebellum
n.r. obscurus	+ + + +		
n.r. pallidus	+ + + 0	+ 0	
n.r. magnus	+ 0 0	+ + 0 0	0 0
n.r. pontis	+		+ + 0 0 0
n.r. dorsalis	+ +		
n.centr.superior	+ + +		0
n.lin.internalis	+ + +		
n.lin.rostralis	+ + + +		

Rostral

Table 1

The table shows the origin of efferents from; +, and the termination of afferents to; 0; the nuclei of the raphe. The relative fibres density is indicated by the number of symbols.

The extremely detailed histological examination of the nuclei of the raphe in the cat has been paralleled by a more superficial study in the rat. Valverde (1962) has described an essentially similar arrangement of the rat brain stem, and has divided the raphe complex into comparable groups. Unfortunately available stereotaxic atlases of the rat brain stem merely subdivide the raphe nuclei into a dorsal and a medial portion.

Employing fluorescence histochemistry techniques, Dahlstrom and Fuxe (1964) have found that 5-HT-containing neurones are almost entirely found within the raphe nuclei. These workers divided the indole-containing areas of the brain stem into 9 groups. All but the two most rostral nuclei contain 5-HT neurones, in addition there are a few scattered 5-HT neurones present around the medial lemniscus, trigeminal nerve and neighbouring structures.

The fluorescence histochemistry technique has also been applied to the investigation of the distribution of monoamine nerve terminals (Fuxe 1965). Using the technique, very fine varicosities (0.5 μ diameter) have been observed within the CNS; in addition, it seems likely that there may exist many other sub-lightmicroscopic varicosities. These varicosities are regarded as being 5-HT-containing terminals since electron-microscopic experiments suggest comparable dimensions for the two structures (Aghajanian, Bloom and Sheard 1969) and electron-microscopic autoradiography indicates

that such terminals are capable of accumulating serotonin-^{3}H (Aghajanian and Bloom 1967). In addition, differential centrifugation experiments have confirmed the localisation of neuronal 5-HT to presynaptic terminals (Michaelson and Whittaker 1962; Ziehr and De Robertis 1963).

5-Hydroxytryptamine-containing terminals are not distributed evenly throughout the cord (Bogdanski, Weisbach and Udenfriend, 1956; Fuxe 1965). There is a high density of terminals in the dorsolateral part of the sympathetic lateral columns, and medium density in the sacral parasympathetic nucleus. 5-HT terminals appear to make synaptic contact mainly with cell processes in the sympathetic columns, whilst most of the parasympathetic innervation appears to be axo-somatic.

Although the dorsal horn contains few 5-HT terminals, those present are arranged in a unique manner. The terminals run parallel, ascending or descending for considerable distances without branching, possibly forming contacts with similarly arranged axons. The ventral horn in the lumbar region differs from other areas of cord in that it has a greater density of 5-HT terminals than catecholamine terminals (Dahlstrom and Fuxe, 1964).

The region containing the ventro and dorso-lateral group of lateral motor cells is more densely innervated than the medial motor region. Small and medium-sized interneurones also receive a 5-HT innervation, although here it appears that most synaptic contacts are dendritic.

The limitations of the technique become apparent when

considering in detail the exact location of the terminals in relation to the motoneurones of the lumbar spinal cord. It is reported that 5-HT terminals make intimate connection with motoneurones (Dahlstrom and Fuxe, 1964), however the technique provides little information concerning the precise position of the 5-HT terminals, nor the nature of the synaptic contact. Using degeneration techniques, these authors have found that the majority of lumbar motoneurones receiving a 5-HT innervation appear to be α-motoneurones. No evidence is available indicating whether the synaptic connections are present on the soma or dendrites of the motoneurones. Whether γ-motoneurones are involved is not known. However it is reasonably clear that if the 5-HT associated with motoneurones represents a neuronal innervation, then the pathway is monosynaptic, and probably descends from the brain stem.

5-Hydroxtryptamine fibres descend in the anterior and lateral funiculi. Terminals in the ventral horn are derived from fibres descending in the medial part of the ventral funiculus and ventral part of the lateral funiculus. The remaining terminals (dorsal horn and sympathetic columns) derive from fibres in the dorsal part of the lateral funiculus.

Although the selective distribution of transmitter material is generally regarded as a prerequisite for transmitter identification, there may be exceptions. However, the fact that there is apparently a systematic arrangement of monoamine terminals throughout the CNS supports the postulate that the monoamine may be involved in synaptic transmission.

Another criterion for transmitter identification involves

the detection of the putative transmitter in extracellular fluid collected from the region of the activated synapse. The theoretical possibility of collecting the released transmitter substances by such gross methods as push-pull cannulae, ventricular perfusion or cortical cups seems remote at first. Even assuming that inactivation processes have been totally suppressed, which is unlikely, the complex arrangement of the neuronal units does not appear to facilitate the draining of transmitter substances from the synaptic cleft.

Two main experimental procedures have been developed to follow the release of 5-HT in brain tissue. Just as in the spinal cord, the forebrain contains many 5-HT terminals and, also as in the cord, these terminals are probably all ultimately derived from 5-HT-containing cells in the nucleus raphe. Aghajanian, Rosencrans and Sheard, (1967) demonstrated that stimulation of the n. raphe in rats caused a reduction in the 5-HT content of the whole forebrain, and a large increase in the primary metabolite of 5-HT - 5-hydroxyindole acetic acid. Using the cup technique, Eccleston, Randic, Roberts and Straughan, (1969) placed cortical cups containing 0.5 ml. of Krebs solution on the pial surface of the cat cerebral cortex. Electrical stimulation of the midbrain raphe, in conjunction with chronic MAOI pretreatment resulted in an increase in collected 5-HT above resting release levels. The cortical cup technique is not a particularly elegant one, mechanical damage caused by the cups to the surface almost certainly results in the local release of many substances from the damaged area; however, it does demonstrate that activation

of the 5-HT-containing cells within the midbrain will increase the release of forebrain 5-HT into the extracellular space. Confirmation of this principle comes from a study in which the n. raphe in rats was electrically stimulated for periods of up to one hour (Aghajanian, Rosencrans and Sheard, 1967).

Fluorimetric estimation of whole and forebrain levels of 5-HT and 5-HIAA indicated a fall (23%) in the former, and a rise (45%) in the latter. These changes may be regarded as being a direct result of raphe stimulation, since stimulation via control electrodes placed in the lateral midbrain evoked no changes in brain monoamine levels.

A comparable study in principle was undertaken by Anden, Carlsson, Hillarp and Magnusson, (1964), in order to demonstrate release from the spinal cord of mice and frogs. Both groups of animals were pretreated with a monoamine oxidase inhibitor 20 hours before the experiment began. The brains and spinal cords were electrically stimulated in vitro for periods ranging from one to two hours. The authors noted a spontaneous release of 5-HT into the Ringer bath solution. Electrical stimulation of the upper parts of the spinal cord markedly enhanced the release of 5-HT. The authors acknowledge the possibility that stimulation of the cord produced damage to the CNS with a subsequent 5-HT leakage. They argue that this seems unlikely since spinal cord levels of 5-HT were usually higher in the stimulated than the unstimulated cords. It seems unfortunate that no attempt was made to confine the electrical stimulation to the 5-HT-containing neurones in the brain stem, but involved the entire upper part of the spinal

cord.

A study employing much more selective stimulation of the brain stem area has apparently confirmed the connection between activity in the raphe nuclei and release of 5-HT from the spinal cord. Electrical stimulation of the raphe nuclei via stereotaxically implanted electrodes in Nembutal-anaesthetised rats for 2 hour periods, has been shown to produce a significant decrease in cord levels of both noradrenaline and 5-HT (Dahlstrom, Fuxe, Kernell and Sedvall, 1965). Changes in levels were recorded in both ventral and dorsal horns. When enzyme synthesis inhibitors were administered, their effect was to markedly augment the reduction in monoamine levels.

Confirmation of the definite existence of a 5-HT pathway descending from the brain stem comes from spinal cord transection experiments. Following transection of the spinal cord, both 5-HT and noradrenaline (in their respective pathways) accumulate in the proximal part of the central monoamine fibre system (Dahlstrom and Fuxe 1965, Carlsson, Magnusson and Rosengren, 1963). In rat, the monoamine build up is evident after about 7 hours and reaches a peak after 24 hours. For up to about 5 days after the transection the distal parts of the transected fibres also demonstrate increased 5-HT levels, indicating that amine synthesis has not been immediately impaired. However, after one week 5-HT levels in the distal cord had been reduced by 85%.

There has been some confusion concerning the possible existence of a 5-HT-containing interneurone in the spinal cord

of the cat. Shibuya and Anderson (1968) note that three weeks after cord transection in cats, lumbosacral segments levels of 5-HT were observed to decrease from 1.36 µg/g to 0.33 µg/g, however the presence of extra-neuronal 5-HT may well explain these apparently high levels. There does not appear to be sufficient evidence upon which to base a hypothesis involving a 5-HT interneurone (cf. Clineschmidt and Anderson 1970).

The experiments described above confirmed the presence of 5-HT terminals in the mammalian spinal cord, and the functional dependence of these terminals upon the level of activity of the parent soma in the n. raphe. They provided, however, no direct evidence suggesting that 5-HT had any pharmacological effect on neurones in the spinal cord. The local exogenous application of drugs from microiontophoresis electrodes can provide information concerning the sensitivity of cells to the putative transmitter. Basically the technique involves the controlled ejection of ions from glass micropipettes placed within the immediate environment of a neurone. (for a more detailed account see 'Materials and Methods').

Although many areas of the CNS have been the subject of microiontophoretic investigations, the cat cerebral cortex, and cat and rat brain stem have attracted the majority of workers. The microiontophoretic application of monoamines onto single neurones in the cat cerebral cortex has produced a series of ostensibly bewildering and contradictory results. Early studies had indicated that microiontophoretically applied

noradrenaline predominantly produced a depression in activity of cortical neurones (Krnjevic and Phillis, 1963). However, these workers had used barbiturate anaesthesia, an agent which was later found to abolish the effects of systemically applied tryptamines (Marley and Vane, 1963). Roberts and Straughan (1968) noted that 57% of cells were excited by NA in the lightly anaesthetised N_2O-Fluothane preparation, the injection of small quantities of barbiturate (thiopentone 2 mg/kg) reduced the excitatory response.

A more elaborate study (Johnson, Roberts and Straughan, 1969), elegantly demonstrated the influence of anaesthesia on cell responses to monoamines. Whilst cortical neurones were predominantly depressed by noradrenaline, 5-HT and isoprenaline in the barbiturate anaesthetised preparation, the situation was reversed in the N_2O-halothane anaesthetised animal, or the encephale isole preparation. Slightly more excitations were observed in the halothane anaesthetised preparation, than in the unanaesthetised preparation, and this may either reflect the poorer condition of the decerebrate animals, or the slightly excitant properties of halothane.

The first study involving the microiontophoretic administration of 5-HT to spinal cord neurones (Curtis, Phillis and Watkins, 1961) reported that the monoamine was ineffective as a modulator of neuronal activity in the barbiturate-anaesthetised cat. In fact application of NA, adrenaline, adenosine triphosphate (ATP), histamine and 5-HT all produced no change in the activity of spontaneously active interneurones. Similarly, these substances, together with acetylcholine (Ach.),

had no effect on extracellularly recorded field potentials evoked by orthodromic or antidromic stimulation. Since in this study iontophoretically applied Ach excited Renshaw cells, presumably the applied substance was reaching the target cells.

Recognising the depressant nature of barbiturate anaesthesia, Engberg and Ryall (1966) repeated the above experiment using decerebrate cats. Both NA and 5-HT were found to be pharmacologically active in the cord. The former reduced the excitability (either spontaneously active or induced firing) of most interneurones and motoneurones. The lack of effect of 5-HT on motoneurones was almost as dramatic as that reported by Curtis (1961). However, Engberg and Ryall (1965) noted that two cells were excited during prolonged application of 5-HT which may indicate that the amount of drug applied was not adequate to influence the activity of the majority of cells, alternatively it is possible that the slight depolarisation caused by 5-HT may not have been sufficient to elicit a detectable response (see Discussion). Confirmation of the depressant nature of iontophoretically applied NA comes from a study carried out on interneurones (Biscoe, Curtis and Ryall, 1966). Noradrenaline depressed the spontaneous and DL-homocysteic acid (an agent which, when applied iontophoretically, excites most cells) - induced firing of 40 interneurones. In accordance with previous work, and in contrast to the findings in the periphery, the two optical isomers of NA appeared to have approximately equal potency as depressants of interneuronal activity.

A parallel microelectrode study (see Materials and Methods)

by Phillis, Tebecis and York (1968) in halothane-anaesthetised and decerebrate cats, further corroborated the depressant effects of monoamines. Both NA and 5-HT frequently elicited a hyperpolarisation of motoneurone membrane potential. Concurrently, antidromic invasion of the soma-dendritic region was abolished. The authors also report that a 'limited number' of interneurones and motoneurones was excited by NA and 5-HT, although no information concerning membrane potential was obtained during such excitations.

These observations of predominantly depressant actions of 5-HT contrast with other reports which note that 5-HT may have excitatory effects. Studies involving unanaesthetised decerebrate cats, (Weight and Salmoiraghi 1966), revealed that out of a group of interneurones, nearly half of which responded to iontophoretically-applied 5-HT, almost three-quarters were excited by the drug.

Further excitatory effects of 5-HT have been reported by De Groat and Ryall (1967). Noting that preganglionic sympathetic neurones in the thoracic spinal cord appear to coincide with a particularly dense accumulation of NA and 5-HT terminals (Dahlstrom and Fuxe, 1965), these workers iontophoretically applied the monoamines to the spontaneously active preganglionic neurones. About three-quarters of all neurones studied increased their spontaneous firing rate when 5-HT was applied. The excitatory responses observed by De Groat and Ryall are especially interesting since sodium pentobarbitone was the anaesthetic employed. In addition the excitatory action of 5-HT was occasionally enhanced when supplementary

doses of pentobarbitone were injected.

Clearly no positive conclusions can be drawn from the iontophoretic experiments. However it is apparent that 5-HT and NA have been pharmacologically demonstrated to have an effect on some spinal cord neurones in a manner similar to synaptic transmitters. A criticism of those papers that recorded the pharmacological activity of 5-HT is that no attempt was made to block the effects with a 'specific 5-HT antagonist' - had this been achieved, the neuronal response could not have been regarded as non-specific. However the problems associated with identifying a 'specific 5-HT antagonis' are great enough to warrant some discussion.

By differentially blocking responses two major types of 5-HT receptors have been identified in the guinea-pig ileum (Gaddum and Picarelli, 1957). D-receptors of visceral smooth muscle are blocked by LSD and its derivatives, whilst M-receptors are blocked by morphine but not LSD.

By fulfilling one of the fundamental requisites of a putative transmitter - the ability to selectively alter the membrane permeability of specific ions - Gerschenfeld (1971) has gone a considerable way towards identifying a possible synaptic involvement for 5-HT in snail (Helix pomatia) ganglion. Two different types of effect have been observed as a result of the action of iontophoretically applied 5-HT. Neurones having 'A' receptors responded with slow, excitatory post-synaptic potentials (EPSP). Two other groups of identified neurones, however, responded in an inhibitory manner. These responses were ionically distinguishable: the 'B' receptor

was regarded as functioning via a selective increase in K^+ permeability, the 'C' receptor increased Cl^- permeability. All three responses were blocked by LSD 25 administered in the bathing medium, some selectivity of effect was demonstrated by the absence of blockade with prostigmine, an anti-cholinergic agent.

The specific blocking of 5-HT responses has also been observed in the mammalian CNS. Bloom, Costa and Salmoiraghi (1964), reported that responses to iontophoretically applied 5-HT and NA were both antagonised by LSD and bromo-lysergic acid (BOL) in rabbit olfactory bulb. Roberts and Straughan (1967) were the first workers to demonstrate the specificity of the LSD antagonism in the CNS. These authors iontophoretically applied four drugs which were regarded as having anti-5-HT activity in the periphery - LSD, BOL (2 brom LSD) methysergide and cinanserin ((2'-(3-dimethylamino propylithio) cinnamanilid hydrochloride) SQ 10643). These drugs reduced or abolished excitations by 5-HT in half the cells studied in the unanaesthetised cat cortex. The specificity of the antagonism was demonstrated by the persistence of the acetylcholine and glutamate induced excitation at a time when the 5-HT response was abolished. The four antagonists were relatively ineffective in blocking 5-HT depressions. In the majority of cells studied, the phenomenon of desensitisation or tachyphylaxis was observed; this is a general characteristic of the action of 5-HT in both vertebrate peripheral systems (Bulbring and Burnstock, 1960) and molluscan (Gerschenfeld and Stefani, 1965) preparations where 5-HT may be an excitatory transmitter.

Confirmation of the specific nature of the 5-HT antagonism by LSD, comes from a report by Boakes, Bradley, Briggs and Dray (1971). An indication of the relative potency (in terms of blocking ability) of the 5-HT antagonists is provided by this report. By far the most potent antagonist was LSD, methysergide was considerably less potent, only blocking about half the neurones responding to 5-HT. BOL, in only blocking about 17% of excitations must be regarded as having negligible blocking capacity. It is however, rather difficult to assess the accuracy of such a potency study, especially as the figures presented in the report raise several questions, (see Discussion). Boakes et al., (1971) also demonstrated that minute amounts (10 - 35 µg/kg) of intravenous LSD also abolished iontophoretically evoked excitatory responses to 5-HT. In a separate study, Bradley and Candy (1970) calculated that the iontophoretically released LSD was present in comparable concentration to the tissue level after the intravenous LSD injection. Some idea of the incredible potency of LSD can be gained when it is realised that brain levels of LSD achieve only 1% of plasma levels (Freedman 1970).

The 5-HT blocking action of LSD has also been demonstrated on cells within the raphe nuclei. These units are characterised by their regular slow firing pattern of 1-2 spikes/sec. Using extracellular tungsten recording electrodes, Aghajanian, Foot and Sheard (1969) noted the total cessation of activity in rat after 10-25 µg/kg LSD had been injected intravenously. Recovery from the inhibition took place gradually and required a period of 15-30 minutes for completion. Non-raphe units

were not affected in a comparable manner, if at all. The authors suggested that LSD may be having a '5-HT-mimicking' action, thus simulating an 'excess 5-HT' situation. The excess 5-HT at the post-synaptic site would initiate a 'compensatory' neuronal feedback mechanism resulting in inhibition of the pre-synaptic unit. The data obtained from the above experiment however, provides no indication as to the mechanism of action of the LSD.

There are several reports in the literature of the apparent direct 5-HT stimulatory action of LSD. Anden, Corrodi, Fuxe and Hokfelt (1968) describe a study in which acutely spinalised rats were injected (intraperitoneal) with either 5-HTP or LSD (0.5 - 2 mg/kg). Both drugs elicited athetoid movements and hyperextension of the hind limbs. In addition similar effects were observed after the pharmacological depletion of the catecholamine system (via α- propyl-dopacetamide H22/54 - a tyrosine and tryptophan hydroxylase inhibitor, and reserpine). The similarity of the gross neurological effects elicited by 5-HTP and LSD at the administered doses, together with the unchanged effects after depletion, suggested to the authors that LSD directly stimulates 5-HT receptors at the post-synaptic site. Extrapolation from gross behavioural observations to speculations concerning synaptic mechanisms must be approached with caution. Implicit in such speculation is the doubtful assumption that both the direct and indirect effect of a drug will be comparable.

Martin and Eades (1969) monitored the electrically evoked flexor reflex of the hind limb in the chronic spinal dog. An

intravenous injection of LSD (15 µg/kg) increased the amplitude of the flexor reflex and evoked the stepping reflex; similar responses were reported with methysergide, (0.06 - 0.25 mg/kg). However, as with the Anden et al., (1968) study, the conclusions to be drawn from the experimental data are strictly limited.

In an attempt to simplify the test situation, Geiger and Cervoni (1958) evaluated the effect of LSD on the monosynaptic and polysynaptic reflex (MSR and PSR) recorded from the ventral roots of cat. Doses (i.v.) below 200 µg/kg had no effect upon the reflex, however the MSR was facilitated maximally with 250-300 µg/kg. It is difficult enough to compare the effects of a drug on different systems when the applied dose is similar, but when the doses differ widely, comparison becomes impossible. This point will be discussed in greater detail later (see Discussion); however when it is appreciated that the psychological effects of LSD are evident in man in doses of about 1 µg/kg, the physiological and pharmacological significance of the above studies can be evaluated.

The biochemical investigation of the effects of LSD on 5-HT metabolism have produced conflicting results. Freedman (1961) notes that LSD (200 µg/kg - i.v.) in rat causes a significant rise in 5-HT and a fall in 5-HIAA, indicating a decrease in 5-HT turnover. These results are not confirmed by a comparable study by Anden et al., (1968). These workers found that in doses below 250 µg/kg i.p., LSD had no effect on 5-HT metabolism, when administered at the dose of 4 mg/kg i.p., there was a slight fall in brain levels of 5-HT.

Thus the mechanism of the LSD-5-HT interaction is far

from clear. Undoubtedly LSD can specifically block the excitant effects of 5-HT, and is capable of modifying neuronal circuits within the CNS. Similarly, even if it were beyond dispute that 5-HT is involved in the behavioural effects of LSD and other psychotomimetics, the nature of this interaction is unresolved.

Thus the studies involving LSD, together with the other experimental data suggest that the 5-HT distributed in the spinal cord is capable of pharmacological activity, however the above experiments give no indication of the physiological significance, if any of 5-HT. The amplitude of the monosynaptic spinal reflex reflects the excitability of the motoneurone pool. Many workers have assumed that changes in amplitude of the MSR in response to the release of monoamines throughout the CNS purely reflect changes in excitability of the pool, however it must be remembered that activity in the spinal (and supra-spinal) neuronal system will also ultimately contribute to motoneurone excitability.

Several investigators have found that injections of 5-HT itself produce a mixture of depression and stimulation of spinal reflexes. Such reports are relatively valueless since 5-HT induces marked blood pressure changes, which are capable of affecting reflex activity, and also the doubt concerning the penetration of 5-HT into the CNS. The application of 5-HTP avoids both these difficulties, and will elevate endogenous 5-HT levels (Bogdanski, Weissbach and Udenfriend, 1958).

The majority of reports concerned with spinal reflex activity have involved decerebrate cat, the MSR and PSR being evoked by

supramaximal stimulation of either the whole dorsal root, or the relevant peripheral nerve. Some workers have monitored the dorsal root potential (DRP) - reflecting pre-synaptic inhibitory events involving the dorsal root afferents.

Banna and Anderson (1968) reported that methysergide and BOL reduced the amplitude of the MSR, PSR and DRP. LSD (300 µg/kg) however increased the MSR - a similar effect to that reported by Geiger and Cervoni (1968). BAS (1-benzyl, 2-methyl, 5-methoxy tryptamine hydrochloride), cinanserin and cyproheptadine had no effect on the response. Since similar results were obtained when either a mid-collicular preparation (in which the raphe-spinal system would remain intact), or a spinal preparation was used, the authors concluded that there was no tonic activity in the raphe-spinal system. The depressant effect of methysergide was attributed to a direct effect on excitability. Apparent confirmation for this interpretation was provided by the results of a depletion experiment; when the spinal cord was depleted of 5-HT, either pharmacologically using reserpine, or surgically (chronic cord section), methysergide still depressed the MSR - presumably in the total absence of 5-HT. However it is probable that even after severe amine depletion physiological transmission remains relatively unaffected, therefore it seems quite likely that the methysergide effect could have been due to anti-5-HT activity. The LSD effect is more difficult to explain, possibly the high dose contributed to the apparently anomalous effect.

As may have been predicted, the intravenous injection of 5-HTP increases the amplitude of the MSR (Banna and Anderson,

1968; Anderson and Shibuya, 1966; Marley and Vane, 1967). The stimulus response latency is decreased by about 0.2 msec. and occasionally spontaneous activity is recorded from the ventral root. The effects become apparent about 2 hours after injection, depending upon the dose of 5-HTP administered, (the dose corresponding to a supramaximal response is 75 mg/kg). It is interesting to note that although the MSR was facilitated (up to 300% above pre-drug amplitude) the MSR and DRP were depressed. Although the qualitative effects of L-tryptophan were similar to those for 5-HTP on the MSR, quantitatively they were much smaller. A more profound difference was noted in connection with the PSR and DRP after L-tryptophan (200 mg/kg), both the PSR and the DRP were increased in amplitude. Methysergide and BOL block both the 5-HTP and L-tryptophan induced excitatory effects (Banna and Anderson 1966), however 5-HTP-induced depression of the PSR and DRP is not susceptible to the 5-HT antagonists. Cinanserin, cyproheptadine and LSD also antagonised the facilitatory effects of 5-HTP, however a very high dose (1.5 mg/kg of LSD) was required before the antagonism was effective.

It seems clear that precursor loading is an effective method of releasing 5-HT from its pre-synaptic terminals (since the effect has a slow onset and there is a marked rise in cord 5-HT levels, Anderson and Shibuya 1966). However the selectivity of the effect is in doubt. It is difficult to explain the opposite effects of 5-HTP and L-tryptophan on the PSR and DRP, possibly the displacement of catecholamines from their pre-synaptic terminals by 5-HTP (Carlsson, Falck, Fuxe

and Hillarp, 1964) may be involved in this process. The reduction of the PSR and DRP by 5-HTP would then be explained in terms of released catecholamine. The relatively small effect of the highly potent 5-HT antagonist - LSD - is also difficult to explain. Banna and Anderson (1966) report that methysergide and BOL are more potent than LSD, 1.5 mg/kg of which was required to block the 5-HTP induced effects.

Some indication of the relative lack of specificity of the antagonists can be gained from a control study involving dopa (L-3,4 dihydroxyphenylalanine). The administration of dopa has been observed to alter the MSR in a manner qualitatively similar to the effects of 5-HTP (Baker and Anderson, 1965). However Banna and Anderson (1966) report that all the 5-HT antagonists, except BAS, were substantially effective in reducing the dopa-induced facilitation. Unless it is postulated that the effect of dopa is mediated via 5-HT, or that the 5-HT antagonists have a direct depressant action, the significance of the above observations is limited. It is unfortunate that the authors did not study the L-tryptophan induced effects in more depth.

Another pharmacological technique which serves to liberate putative transmitters from their pre-synaptic terminals involves reserpine treatment. Reserpine blocks monoamine transmission by depleting transmitter stores - the initial effect is to release the transmitter; when used in conjunction with a MAOI, the effect is comparable to that produced by dopa and 5-HTP (Anden Jukes and Lundberg, 1964). The technique does of course involve the release of both 5-HT and NA, it is theoretically

possible to differentiate between the effects caused by the two monoamines by the use of specific antagonists.

It has been demonstrated that dopa and 5-HTP inhibit transmission from the FRA (flexor reflex afferents = Group II and III muscle afferents, cutaneous afferents and high threshold joint afferents. (Lundberg 1966)) to motoneurones, primary afferents and ascending pathways (Anden et al., 1964), a similar effect is observed in the decerebrate cat (Eccles and Lundberg 1959; Holmqvist and Lundberg, 1961; Carpenter, Engberg, Funkenstein and Lundberg, 1963; Holmqvist, Lundberg and Oscarsson, 1960). The contribution to this inhibitory state by 5-HT has been demonstrated to be small but significant. Both BOL and methysergide provided a partial release of reflex transmission from the skeletal rigidity associated with decerebration, in addition the administration of nialamide (a MAOI) enhanced this tonic control (Engberg, Lundberg and Ryall 1967).

The report (Engberg et al., 1968) also provides the first evidence for a physiological role for the raphe nuclei. The nuclei were totally destroyed by electrolytic lesions placed upon the midline, the effect of the lesion was to partially reduce decerebrate inhibitory control of transmission from the FRA, however the authors suggested that the raphe nuclei may normally only be partially responsible for decerebrate control. Coincident damage to the medial reticular formation may be equally responsible for the observed effects.

A non-pharmacological method for releasing monoamines is to electrically stimulate the descending pathway. The problems associated with selectively stimulating fine monoaminergic

pathways (Lundberg 1966) probably account for the paucity of reports employing the technique.

By electrically stimulating the caudal raphe nuclei, Clineschmidt and Anderson (1970) were able to modify the spinal MSR. Both depressant and excitatory effects were observed, depending upon the stimulus parameters. Inhibitory effects, which were usually recorded when the MSR was evoked 25 msecs. after the end of the conditioning train applied to the brain stem, were blocked by both LSD (250 - 500 µg/kg), BOL and methysergide. Excitatory effects were unaffected by the 5-HT antagonists. By selective perfusion of spinal cord and brain stem areas, the authors suggest the spinal cord as the site of the antagonism.

Analysis of conduction velocities led them to predict the existence of a 5-HT-containing interneurone in the spinal cord. This postulate however, is in direct conflict with all the evidence provided by the fluorescence histochemists, (Dahlstrom and Fuxe 1964 etc.), who have consistently reported that all 5-HT-containing cells are restricted to the brain stem area. The authors do not state whether the descending inhibitory influence was blocked with doses of LSD below 250 µg/kg, since at such doses the LSD effect may well not be specific.

There is thus considerable evidence implicating 5-HT in a neurotransmitter or neuroregulatory role in the spinal cord. Fluorescence histochemistry has demonstrated its distribution within the cord, and brain stem and the dependence upon raphe nuclei activity has been confirmed. Iontophoresis experiments have produced confusing results, although there is evidence

suggesting that 5-HT may have both inhibitory and excitatory effects in the cord. The confusion concerning the concentrations at which an antagonist may be specific has limited the significance of some antagonism studies in the cord. Similarly, more recent experiments have highlighted the problems associated with pharmacologically releasing monoamines from their pre-synaptic terminals. However, 5-HT does appear to have a physiological action in the cord, and there is general agreement that it can increase motoneurone excitability. Its effects on inter-neuronal systems are far from clear, although the indoleamine may be responsible for the tonic inhbition from FRA. The only attempt to avoid the hazards of pharmacological release of monoamines, by electrically stimulating the 5-HT-containing cell bodies, proved disappointing - the apparently inhbitory nature of the descending effect on the motoneurone pool conflicts with previous reports.

It is now evident that several of the more important criteria involved in the identification of a central nervous system transmitter have been met: 5-HT does appear to be a potential transmitter. More positive identification can only be gained by strictly comparing the effects of the endogenously released drug, with those of the local exogenous application. For a drug to have any effective neuropharmacological action, it must ultimately modify the excitability of the 'target' cell. In some instances this will result in a change in neuronal firing. If this is the case, then the potential mimicking action of the exogenous agent can be examined. Once it has been established that the qualitative effects of the two agents are similar, and it is known that both agents are acting via

a post-synaptic mechanism, then the test of pharmacological identity may be applied. Briefly, the theory states that agents which interact with the natural transmitter, should interact with the suspected (exogenously applied) transmitter in a similar way. There are, in addition, several properties which characterise the mechanism of the synaptic action which must be proved to be identical for the exogenous and endogenously released transmitter. (see Discussion)

The most satisfactory method of releasing transmitter substances from their pre-synaptic terminals is to electrically excite the parent somata. Having succeeded in releasing the transmitter substance, the aim of the project was to attempt to mimic the post-synaptic effect and to block the effect with a specific antagonist.

The first series of experiments involved the characterisation of the post-synaptic response to stimulation of the 5-HT-containing cell bodies in the nuclei raphe. Intracellular microelectrodes were inserted into spinal motoneurones to record the post-synaptic events. The problems associated with intracellular recording and the concomitant extracellular iontophoretic application of drugs prompted a study involving a more stable test system.

The descending raphe-spinal influence on spinal reflexes has been studied in detail. The effect of 5-HT antagonists and precursors on this control system has been investigated. This involved the systemic administration of drugs, a technique which provides no information concerning the site of action of the drug.

Finally, the raphe-spinal influence on spinal reflexes has been monitored using microelectrodes. Changes in excitability of the motoneurone pool have been observed after raphe nuclei stimulation. Such changes have been modified or mimicked by the iontophoretic application of various drugs into the environment of the motoneurones. These observations have resulted in a discussion concerning the possibility and nature of 5-HT-mediated synaptic transmission in the spinal cord.

M A T E R I A L S A N D M E T H O D S

MATERIALS AND METHODS

Experimental animal

The project involved a total of 40 cats and 190 rats. The development of different techniques during the project has meant that slight variations on the basic preparation have been employed. However these are all based upon the necessity to monitor the electrical activity of spinal motoneurones during stimulation of the brain stem nuclei of the raphe.

The initial experiments involved developing the technique of intracellular recording together with the manufacture and use of the parallel microelectrodes. The rat was therefore the animal of choice in these early stages. However cats were experimented upon when difficulty was experienced in obtaining stable intracellular recordings with parallel microelectrodes. The added stability of the cat spinal cord enabled recordings to be obtained using this type of electrode. Antagonism studies involving the systemic administration of drugs whilst either recording from single motoneurones or recording monosynaptic reflex activity were also satisfactorily accomplished using rats.

Preparation of both types of experimental animal was similar. The experimental procedure relating to the rat will be described in detail, reference being made at relevant points to variations in procedure involving cat.

Albino Wistar rats of both sexes were used throughout the experiments. The optimum weight appears to be 300-450g. Heavy old animals were avoided since the bone of the vertebrae

tends to be harder and more prone to prolonged haemorrhage. Younger, lighter animals were difficult to work with since the level of the lumbar enlargement is very variable; in addition, the younger animals did not tolerate anaesthesia and the considerable surgery for a sufficient length of time. Male and female cats, between 2.5-3.5 Kg., were used. Animals experiencing respiratory difficulties were not used.

Anaesthesia

A general anaesthetic will necessarily modify activity within the nervous system. Thus if a decerebrate, unanaesthetised preparation is unsuitable, the effect of anaesthesia on the recorded nervous activity must be taken into account.

The depressant effects of barbiturates on nervous activity are well documented. French et al., (1953) noted that both pentobarbital and ether depressed evoked potentials in the reticular formation and blocked electroencephalographic arousal produced by reticular stimulation. The profoundly disrupting effect of barbiturates on iontophoretically evoked monoamine responses in the cortex has already been discussed, (Roberts and Straughan, 1967, see Introduction).

The effect of halogenated gaseous anaesthetics has been investigated on mono- and polysynaptic pathways in the spinal cord (De Jong et al., 1968). These authors found that, together with the halothane anaesthetics, cyclopropane and ether exerted an approximately equal depressant effect on monosynaptically and polysynaptically transmitted reflex potentials. These observations corroborated similar observations made with barbiturates (Esplin, 1963; Austin and

Pask 1952; Løyning et al., 1964). Barany (1947) had earlier suggested that the observations of French et al., (1953) could be explained in terms of the depressant effect of centrally acting anaesthetic drugs increasing in a geometric fashion with the number of synapses in the pathway. However, an alternative explanation, and one which is more acceptable since it is compatible with more recent reports, (De Jong et al., 1968) is that each synapse is affected to the same extent by the anaesthetic. Thus, provided the depression induced by anaesthesia does not exceed the capacity of the synapse to transfer an impulse, transmission should occur as successfully across several synapses as it does across a single synapse.

A constant level of anaesthesia is extremely important when monitoring excitability of the nervous system. It is essential to be able to attribute changes in neuronal excitability to the effects of administered drug rather than a change in the level of anaesthesia. Fluothane, a gaseous anaesthetic, can be delivered in a very precise and controlled manner via a Fluotec dispenser. This property suggested Fluothane as the anaesthetic of choice. A gaseous anaesthetic also has the advantage that the level of anaesthesia may be altered relatively easily and in a controlled fashion.

Another important property of Fluothane is the apparent lack of effect on monoamine systems. Experiments involving the iontophoretic application of monoamines onto cells in the cerebral cortex of cat (Roberts and Straughan 1967) indicate that while barbiturate anaesthesia is responsible for modifying

the responses of cells to the applied monoamines, Fluothane appears to interfere minimally with this process.

Since there is still the possibility that Fluothane may well be masking some effects and inducing others, a series of experiments involving decerebrate preparation was also undertaken. Unfortunately decerebrate preparations, although being free of anaesthetic usually tended to have rather poor, fluctuating blood pressures and frequently required artificial respiration. Thus although these experiments provided a series of controls for the Fluothane preparations, difficulties associated with the decerebrate animals precluded their more general use.

Induction

Fluothane was used both to induce and to maintain anaesthesia. Induction was achieved by placing the rat in a polythene box with an inspection window. Anaesthesia was induced in cats by placing a mask over the head of the animal. Oxygen was the vehicle for the administration of the Fluothane.

Maintenance

The maintenance level of Fluothane varied from one animal to another in the range $0.\frac{5}{9}$-0.9%. During surgery the animals were more deeply anaesthetised than at other times, as a consequence no experimental data was collected for at least 3 hours after the surgery had been completed. This time interval was sufficient for the animal to stabilise at a lighter level of anaesthesia. This depth of anaesthesia however, was sufficient to abolish the flexion reflex in response to a mild squeeze of a paw. The level of anaesthesia

in a rat is not easy to judge. However by considering the respiration rate and volume, electrocardiogram and blood pressure, a reasonably accurate assessment could be made.

Decerebrate Preparations

In an attempt to assess the possible effects of Fluothane on the recorded responses, a series of 20 unanaesthetised decerebrate rat preparations were performed. Under general anaesthesia the carotid arteries were tied off just before a mid-collicular lesion was made. Great care was taken when performing the lesion since the raphe nuclei lie just posterior to the colliculi. Immediately after the lesion the blood pressure fell rapidly, frequently the animal was respired artificially. In some cases the rat died within 30 minutes of performing the decerebration, in others the blood pressure recovered and on some occasions the rat lived for up to 5 hours. In many but not all animals, evidence of muscular rigidity was apparent within 30 minutes of terminating the anaesthesia. In each case the completeness of the section was checked histologically at a later date. In some animals (both decerebrate and anaesthetised), Flaxedil was administered (under supervision) to reduce spontaneous muscular activity. The relaxant was also administered to those preparations in which respiratory movements severely limited the stability of the recording situation. Flaxedil was only adminstered to animals whose depth of anaesthesia had been constant over a period of at least one hour.

Surgery

Preliminary procedure

After induction, rats were placed on an electrically heated operating table. At this point anaesthetic was delivered via a mask over the head of the animal. The animal having been shaved, a polythene cannula with a specially constructed glass tip was inserted into the trachea.

The femoral vein and artery were cannulated. Throughout each experiment blood pressure was continuously monitored via an arterial cannula filled with Heparin (Pularin 200 i.u. per ml). A two way tap was attached to the end of the cannula (Portex-nylon intravenous cannula, 30 cms blue luer 200/300/020). Although few workers have continuously monitored blood pressure from rat, the above described procedure presented few problems. No record of the number of successful recordings have been made, however about 80% of experiments were accompanied by a continuous recording of blood pressure.

The femoral vein was cannulated using a slightly larger cannula (Portex-nylon intravenous cannula, 30 cms pink luer 200/300/030), a two way tap was attached to the end of the cannula to allow the permanent infusion of a drip or the periodic administration of drugs.

A similar procedure was adopted for experiments involving cat, except that the larger cannula was used for both the vein and artery.

Laminectomy

A longitudinal skin incision was made in the animal's dorsal surface from L3 to L7. The superficial muscle layers were cut using a scalpel. Deeper layers were carefully cut with a pair of fine, sharp scissors. There was very little

blood loss during this procedure. As far as possible the spines and dorsolateral surfaces of the vertebrae were scraped clear of muscle. The animal was then transferred to the stereotaxic frame. A pair of specially fashioned spade-ended clamps were passed through the skin at each side of the animal, rigidly clamping onto the sides of the vertebrae. A second vertebral clamp attached to a central spine increased the immobilisation of the vertebral column. The pelvic girdle was supported from underneath the animal, the tail being attached to the end of the frame. Stable intracellular recordings can only be made when the relevant section of cord is located between vertebral clamps. Thus it was essential to position the lumbar enlargement, which in rat is located between L.5-L.7, between these clamps. The dorsal and latero-dorsal aspects of at least three lumbar vertebrae were removed using bone nibblers. Occasionally a small haemorrhage was associated with this operation. Persistent bleeding was arrested either with topically applied thrombin, or pressure. Having removed the bone, the side flaps of skin were tied to form a pool which was subsequently filled with saline at $37 \pm 1^{\circ}C$.

With the aid of a Zeiss binocular dissecting microscope an incision was made in the dura. In earlier experiments, an anterior-posterior longitudinal slit was made, the dura being deflected back on either side and pinned with entomological pins. Subsequent experiments indicated that better spinal cord stabilisation was achieved if the dura was left substantially intact, incisions being made only where necessary. From this point great care was taken to keep the cord moist, warm saline being applied via a dropping pipette.

Using two suitably fashioned glass probes, two or three ventral roots were very carefully eased from under the spinal cord. It is essential when preparing the ventral roots for either recording or stimulating, that they are not damaged in any way. The roots were cut using iris scissors and laid upon silver stimulating electrodes. Corresponding dorsal roots were prepared similarly in those experiments where the monosynaptic response (MSR) was being monitored. When the surgery associated with the spinal cord was completed, the saline in the pool was replaced with liquid paraffin B.P. at $37 \pm 1^{\circ}C$.

Stimulation of the raphe nuclei

Co-ordinates.

Although the raphe nuclei have been studied in both rat and cat and subsequently sub-divided on a histological basis, the positions of the individual nuclei are only described stereotaxically in an atlas of the cat brain (Snider and Niemer, 1961). The co-ordinates corresponding to nucleus raphe magnus and nucleus raphe pallidus were chosen as the relevant nuclei of the raphe to stimulate, since these two nuclei predominantly project to the spinal cord (Brodal et al., 1961). The exact stereotaxic co-ordinates in cat are: AP-6.0 mm; LO.0 mm; H-9.5 mm. The presence of a bony tentorium in cat prevents the vertical placement of stimulating electrodes within the raphe nuclei. Electrodes were thus implanted parallel to the tentorium. Fig. 2 illustrates the position of the nuclei of the raphe in cat and the arrangement of stimulating electrodes along the midline.

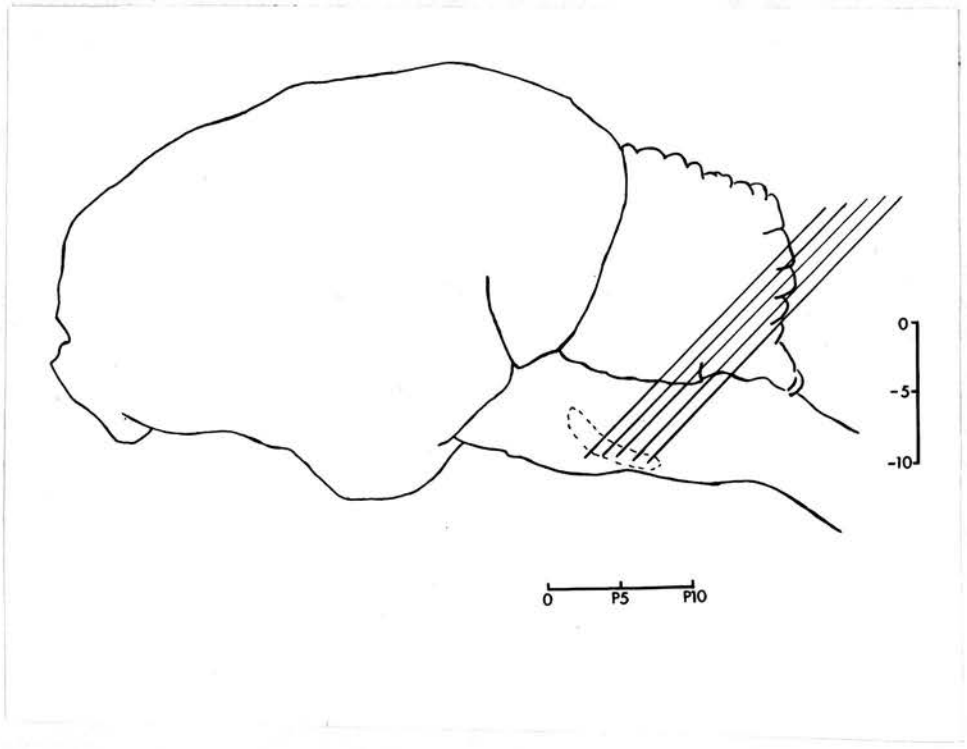

FIGURE 2

Approximate position of raphe nuclei (indicated by dotted line) in the midline of the brain stem of cat (from Snider & Niemer). The diagram also indicates the arrangement of monopolar stimulating electrodes along the midline.

Stereotaxic atlases relating to rat brain merely divide the raphe into a dorsal and medial component. The co-ordinates relating to the more caudal median raphe have been adopted: AP + 0.35; L 0.0; H -2.6 (Konig and Klippel 1963). The position of the raphe nuclei in rat brain is shown in Fig. 3. Since the stereotaxic frame used for experiments involving rat is based on the De Groot system, corrections to the Konig and Klippel co-ordinates were necessary.

Stimulating Electrodes

In all experiments involving rat, concentric bipolar stimulating electrodes have been used to electrically excite the raphe nuclei. Bipolar electrodes are larger than the monopolar type and thus cause slightly more tissue damage. However the advantage of relatively restricted current spread associated with bipolar electrodes was judged to outweigh the disadvantage of greater tissue damage.

Stimulation parameters

In preliminary experiments, the stimulation parameters were varied. However optimum stimulation parameters were identified, by an examination of motoneurone responses and in the majority of experiments stimulation of the brain stem was effected using 100 msec. trains of stimuli at 100 Hz. Each square-wave pulse was 0.1 msec. in duration. Intensities of stimulation varied from one experiment to another, but never exceeded 0.25 mA.

Bipolar stimulating electrodes

Concentric bipolar stimulating electrodes were constructed from stainless steel tubing, external diameter 0.016 in.,

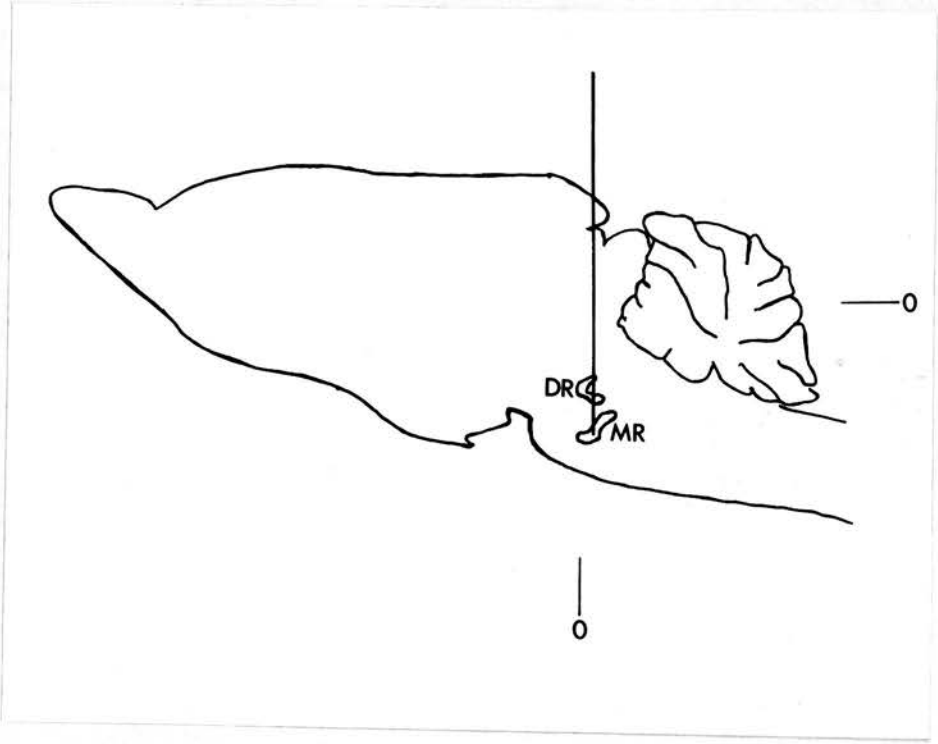

FIGURE 3

Position of dorsal and median raphe nuclei in the midline of the brain stem of rat. The figure is based upon the stereotaxic atlas of Konig & Kippel (1963). The position of the sinlge bipolar stimulating electrode is indicated.

internal diameter 0.006 in., cut into 2.5 inch. lengths (personal communication. M. H. T. Roberts). The centre electrode consisted of a diamel coated stainless steel wire 0,004 in. in diameter. After being coated with undilute Araldite varnish (epoxy resin PZ985), the wire was fed into the tubing. The steel tubing was then coated with dilute Araldite varnish and baked at 200°C for ten minutes. Four additional coats were subsequently applied. Having tested the electrode to ensure complete electrical isolation between the inner and outer electrode, the inner wire was cut at one end so that it projected about 1 mm. beyond the outer tube; the end was then fashioned using a very fine grind wheel, to those dimensions shown in the diagram (Fig. 4).

Leads from the stimulating unit were soldered to the upper end of the electrode. The electrodes were only used when the electrical resistance, as measured in normal saline via a wheatstone bridge, was between 25 and 50 KΩ.

Monopolar stimulating electrodes

One series of experiments involved the mapping of the anatomical position in the brain stem of cat from which post-synaptic motoneurone responses could be elicited. Five tungsten electrodes 2.0 mm. apart arranged in a row, were lowered systematically either in the midline or across the brain stem. The evoked responses from different regions of the brain stem were examined by systemetically stimulating within the brain stem region.

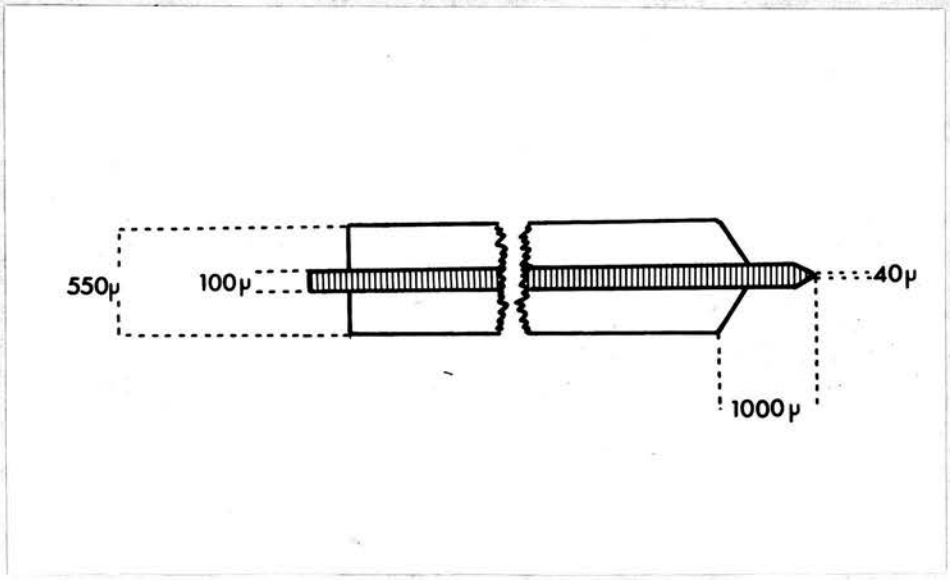

FIGURE 4

This diagram represents the approximate dimensions of the bipolar concentric stimulating electrodes used in the experiments involving rat.

The manufacture of monopolar stimulating electrodes was adapted from a published report (Hubel 1957) concerning the manufacture of tungsten recording electrodes. The electrodes were made from 0.5 mm. diameter tungsten wire. Using a saturated solution of ferrous sulphate as the electrolyte, the tungsten wire was electrolytically etched until the tip diameter was about 40 μ. Care was taken to keep the etching current very low, thus avoiding 'pitting' of the suface. By moving the electrode slowly, in and out of the electrolyte, a gradual taper of the tip was achieved. Electrode insulation to within 500 μ of the tip was achieved by applying dilute Araldite varnish with a paint brush, the operation taking place under a microscope.

Monitoring of physiological parameters

Blood pressure was continuously recorded via a Bell and Howell pressure transducer. A permanent record was made on one channel of a Devices M4 Polygraph recorder. The same instrument was used to provide a record of the electrocardiogram. The relative change in shape and amplitude proved to be a very important guide to the state of the animal. On occasions, the QRS complex reversed completely (a clinical feature of cardiac infarct) and this invariably heralded the death of the animal.

Temperature Control

The body temperature of anaesthetised rats was observed to fall steadily unless an attempt was made to maintain it artificially. An indication of the body temperature was given by the rectal temperature which was regularly recorded through-

out the experiment. During the surgery the animal lay on a heated operating table. Once in the stereotaxic frame, a heated blanket was arranged underneath the animal. The temperature of the blanket was thermostatically controlled via an automatic feedback unit based on the design of Pokrousky (1960) and involving a heat sensitive OC71 transistor placed in the animals rectum. The heating blanket was usually sufficient to maintain the body temperature at $37\pm1^{\circ}C$. Additional radiant heating could be provided if necessary by a lamp directed on to the animal.

The temperature of the paraffin pool was maintained at $37\pm1^{\circ}C$ by means of a small heating element submerged in the pool. The heating element consisted of 21 cms. of nichrome resistance wire wound round a piece of capillary tubing. The element was then sealed in a second length of glass tubing; the heater current was controlled by a unit similar to that used for the heating blanket.

Intravenous Injections

In an attempt to conserve the blood volume between 0.5 and 1.5 ml. of injection saline was administered i.v. to those rats that lost some blood during the surgery.

Cats received a continuous infusion of 'cocktail'. Although the proportion of ingredients was occasionally varied, the following is the basic recipe (J. Manson - personal communication).

Isotonic glucose	5.34 gm.
Insulin	1 unit.
Penicillin	1 million units.

Distilled water (Pyrogen free) 100 mls.

Macrodex-Rheomacrodex (1:1) 50 mls.

Macrodex is a high molecular weight ($\bar{M}$W. 70.000) solution employed clinically to promote renal function and conserve blood volume. In later experiments the use of Rheomacrodex was extended and eventually replaced Macrodex. Rheomacrodex has a lower molecular weight ($\bar{M}$W 40,000) than Macrodex and is designed to promote blood flow and reduce erythrocyte aggregation. It has been difficult to quantify the efficacy of these additives, however there are indications that Rheomacrodex tended to prolong the useful life of the preparations.

In an attempt to reduce inflammation and general reaction to the surgery, hydrocortisone sodium succinate (50 mg) was added to the drip. The infusion rate was between 2-3 mls per hour.

Experimental procedure - cat

40 cats of either sex, between 2.5-3.5 kg, have been used. The majority of animals were induced and maintained on Fluothane. In 6 animals pethidine 4 mg/animal was injected i.p. an hour before the surgery began. These cats were anaesthetised by an i.v. injection of thiopentone, the tissue around the incision having been first locally anaesthetised with lignocaine. On completion of the surgery the animal was maintained on Fluothane. In this way it was hoped to avoid the partially hypotensive effects of Fluothane experienced during surgery. The technique was never found to be satisfactory, a stable level of anaesthesia later on in the experiment was difficult to achieve since the effects of the

barbiturates were long lasting.

Another problem associated with the long term use of Fluothane is increased cerebrospinal fluid (CSF) production. On occasions it was noted that ventral roots from which recordings were being made became enclosed in a layer of CSF. The effect of this layer was to shunt both the biological and the non-biological signal. In some preparations the CSF could be sucked away from the spinal cord using a sucking pipette; however a few preparations, invariably rats, were abandoned because of the continuous flow of CSF.

The preliminary surgery was similar to that for the rat. A laminectomy was performed between L4 - L7, the animal then being clamped into a stereotaxic frame based on the Eccles model. The third lumbar ventebra together with the pelvic girdle were firmly clamped. A further clamp was attached to the central spine of a thoracic vertebra. Little difficulty was experienced in keeping the body temperature of cat to $37\pm1^{o}C$.

Recording electrodes

Probably the simplest method of recording bioelectric activity within the CNS is to place a metal wire or needle in the vicinity of a neurone. However, due to such factors as junction potentials, electrical insulation and tip configuration, metal microelectrodes are unsuitable for intracellular recording. By using an electrolyte filled microelectrode, a bridge is formed between the metal (silver) wire inserted into the electrode and the biological tissue. The measured potential will now be the sum of the membrane potential and a potential generated between the electrolyte, the glass of the microelectrode

and the intracellular contents. This potential - the tip potential - varies between -2 to -5 mV for recently filled 0.5-1.0 μ tip diameter 3M KCl-filled electrodes (Levine, 1966). The generated potential has been attributed to hydration of the glass (Agin, 1969). Since hydration is a function of time and temperature (Holland, 1964), glass microelectrodes older than five days were not used. Similarly, the filling temperature is invariably kept at a minimum to reduce the development of a tip potential.

Microelectrode glass preparation

A problem often experienced with microelectrode recording is that of abnormally high electrode resistance (i.e. greater than about 15 MΩ). Often the resistance can be substanially reduced either by passing a current pulse down the electrode or by gently tapping the microelectrode holder. However electrodes exhibiting consistently high recording resistances were rejected. High electrode resistance is often due to foreign matter blocking the electrode tip. Thus every precaution was taken to ensure that perfectly clean glass was used to make electrodes. All glass microelectrodes were made using Pyrex glass of external diameter 1.5 mm and internal diameter 1.0 mm. The tubing was cut into 10 cm. lengths and completely immersed in concentrated nitric acid for several hours. The glass was then thoroughly washed in double distilled water and finally placed in absolute alcohol. When removed from the alcohol, the glass was allowed to dry and was then ready for 'pulling'. A similar type of glass was used for the construction of multi-barrelled micropipettes or microelectrodes. A 'blank' was

manufactured by securing five glass tubes with a brass collar at each end, a technique developed by Herz et al., (1965). After applying Araldite adhesive around the collar, the blank was laid on a flat plate deeply grooved to ensure that the two collars lay exactly parallel and baked at 200°C for 20 minutes. Cleaned glass was wrapped in polythene and stored in a dust-proofed container. Unfilled, pulled electrodes were stored tip upwards, thus reducing the chance of (dust) blocking the tip.

Pulling Glass Microelectrodes

Single Barrel Electrodes

A vertically arranged microelectrode puller has been used throughout these experiments. Capillary glass has been drawn out into fine microelectrodes by heating the glass tube over a length of 5 - 11 mm. When soft the glass was quickly drawn apart to form two electrodes. Fig. 5 indicates the accepted nomenclature relating to microelectrodes.

Electrode shape is dependent upon many factors - probably the most important being the heating coil characteristics and the type of glass tubing used. By increasing the temperature and size of the coil, it is possible to increase the length of the microelectrode shank and reduce the tip diameter. The electrical characteristics of the heating coil changed with use - the resistance increasing; it was thus necessary to increase the coil current over a length of time in order to maintain a particular electrode shape.

The shape of the tip is particularly important, during the course of the project is has been observed that more stable intracellular recordings can be obtained from electrodes whose tips and shanks have a gradual taper.

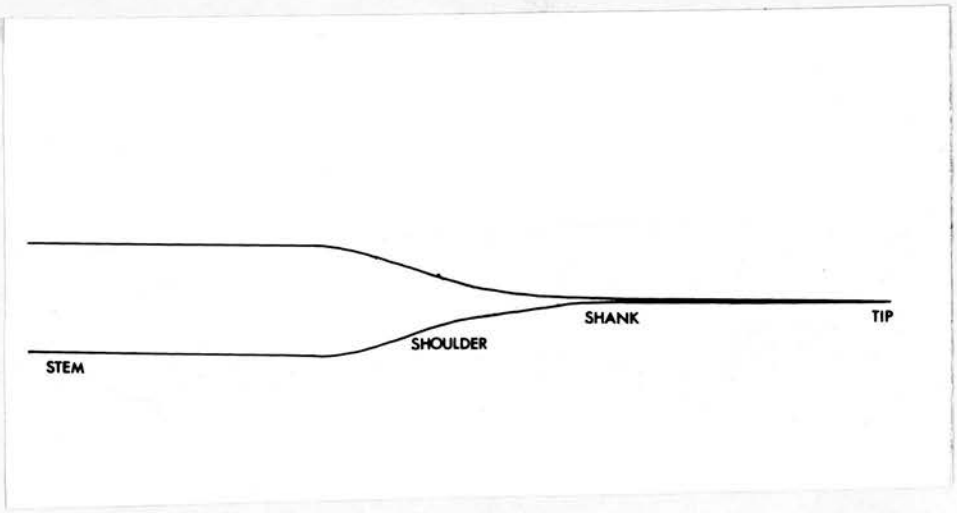

FIGURE 5

Nomenclature relating to glass recording microelectrodes.

Conically shaped tips often critically damage the neurone membrane. The extra damage associated with the conical tip may be attributed to the larger mass of this type of tip. Attempts were made to modify the single barrel electrodes for intracellular recording with the aid of a Fonbrune micro-forge. Using the incorporated microscope, a 0.5 g. weight was attached to a small hook fashioned at the end of the pulled electrode. By slowly moving an electrically heated element along the shank of the electrode, a finer, more parallel shank was produced. This procedure was difficult to develop and extremely time consuming. Since these modified electrodes did not result in longer or more stable intracellular recordings than those provided by the conventional type, the modifications were abandoned.

Multibarrelled iontophoresis electrodes.

Five-barrelled electrode blanks were pulled on the same electrode puller as was used for the recording electrodes. A larger heating coil was used, both to provide more heat, and to soften a larger area of the electrode. Tne blank was first fixed to the puller in such a way that the upper brass collar was firmly clamped in the upper chuck.

The heating coil was then arranged so that it coincided with and surrounded, the middle of the blank. Having turned on the coil current and softened the glass tubes of the blank, the lower chuck was raised and clamped around the lower brass collar. By not clamping the lower collar until the centre of the blank was softened, any strain due to a slight curvature of the blank was avoided.

The glass tubes at the centre of the blank became fused together when the lower chuck was rotated twice through 360°. Immediately, the lower chuck was lowered by about 1 cm. and the heating current turned off. The coil was adjusted so that it corresponded to the fused section of electrode and again turned on. As a result, the electrode again softened, and eventually the lower half of the blank dropped down. The upper section of the electrode was immediately raised above the heating coil. Although some authors (Curtis, 1964) advocate the use of an additional pulling force involving a solenoid, when pulling microelectrodes with the present puller this procedure was not found to contribute to the process. It was often the case that only one usable electrode could be obtained per blank. A frequently encountered problem associated with pulling multibarrelled electrodes was that the shank was too short. Obviously, this could be partially overcome by increasing the temperature of the heating coil, however there was a critical temperature beyond which the electrode shank became curved. Occasionally the barrels of the electrode did not fuse properly (this was evident when the electrodes were examined under the microscope); such electrodes were discarded.

Filling microelectrodes

Electrodes were originally filled in several stages. Firstly, by boiling under reduced pressure in methanol, then replacing the methanol with distilled water and subsequently introducing the electrolyte. The later stage involved removal of most of the water in the electrode, using a 1 ml.

syringe and needle with a length of fine polythene tubing attached. The water was then replaced by the electrolyte. About 24 hours was required for diffusion of the electrolyte to the tip, consequently electrodes were not used for at least a day after manufacture. This rather lengthy procedure was designed to reduce the possibility of damage to the electrode tip during the filling stage.

Since each manoeuvre increased the likelihood of dirt being introduced into the electrode tip, the above method was modified. The majority of recording electrodes were filled directly with 3M potassium chloride. Great care was taken to remove any particles from the solution by vacuum filtration through a 3 micron filter (Whatman Grade 03). The empty microelectrodes were filled by boiling under reduced pressure at or below 60°C. Electrodes were normally filled after about ten minutes boiling.

A second modification allowed electrodes to be made immediately before use. An electrode was broken back, so that it had a tip diameter of about 1.5 - 2.5 μ. The barrel of the electrode was then filled with electrolyte. An electrolyte-filled syringe was attached to the electrode by means of a piece of polythene tubing, which just fitted over the shank of the electrode. Then, by forcing electrolyte from the syringe into the electrode, the solution entered initially under pressure, subsequently via capillary action. Descriptions of this method have been reported in the literature (Frank and Becker, 1964) however the technique was developed independently by the author. Although the published account indicates

that only electrodes with tip diameters greater than about 5 μ are amenable to this method of filling, no difficulty has been experienced filling electrodes with smaller tips.

Occasionally recording electrodes were filled with potassium citrate. Since potassium citrate tends to interact comparatively rapidly with the glass at the tip of the electrode, these electrodes were kept only for two days after manufacture.

Immediatley before use, the tips of the unbroken microelectrodes were touched against a glass probe under a microscope so that the tip diameter was between 1.5 - 2.5 μ. Although theoretically the tip of an intracellular recording microelectrode should be as small as possible causing minimal damage to a cell membrane, the lower diameter limit is set by the electrical resistance of the electrode. Thus a 1.5 - 2.5 μ tip has a resistance in 3MΩsodium chloride of between 3-10 MΩ, a smaller tip has a correspondingly higher resistance which is unsuitable for recording membrane events and unnecessary when considering the large cells from which recordings were being made.

Multibarrelled electrodes were first filled with distilled water by boiling under reduced pressure. Having examined the tips to ensure that filling was complete and that the tip was unbroken, as much distilled water as possible was removed from each barrel. After being centrifuged for 10 minutes at 2,000 r.p.m. to remove any particles the drug solution was introduced into the electrode, care being taken to avoid entry of small air bubbles into the shank, which may have impeded diffusion of the drug into the water-filled tip. Electrodes

were filled with drugs about 24 hours before use and normally stored refrigerated for a maximum of 5 days.

Compound Microelectrodes

The activity of cells which spontaneously produce action potentials is normally studied with techniques of extracellular recording. Changes in the frequency of the extracellularly recorded action potentials will reflect fluctuations in membrane potential. However neurones which are normally silent under experimental conditions may be undergoing considerable fluctuations in membrane potential whilst not producing an action potential. Since the only manifestation of this activity is the change in membrane potential, it is necessary to record this event directly with an intracellular electrode. In addition to providing information concerning membrane potential, intracellular electrodes can be used to measure membrane resistance and reversal potentials during synaptic action.

Basically, two types of electrode have been developed allowing simultaneous intracellular recording and extracellular iontophoresis. The co-axial electrode consists of an inner recording electrode projecting 40-60 μ beyond an outer iontophoresis barrel. Capacitative coupling between the recording and iontophoresis barrels is a limiting problem with this type of electrode. A more successful form of 'compound microelectrode' is the parallel assembly consisting of a recording barrel projecting between 30-60 μ beyond the iontophoresis barrels and externally glued to it.(Fig. 6)

Developing the technique for making parallel microelectrodes took several months. The main difficulty was

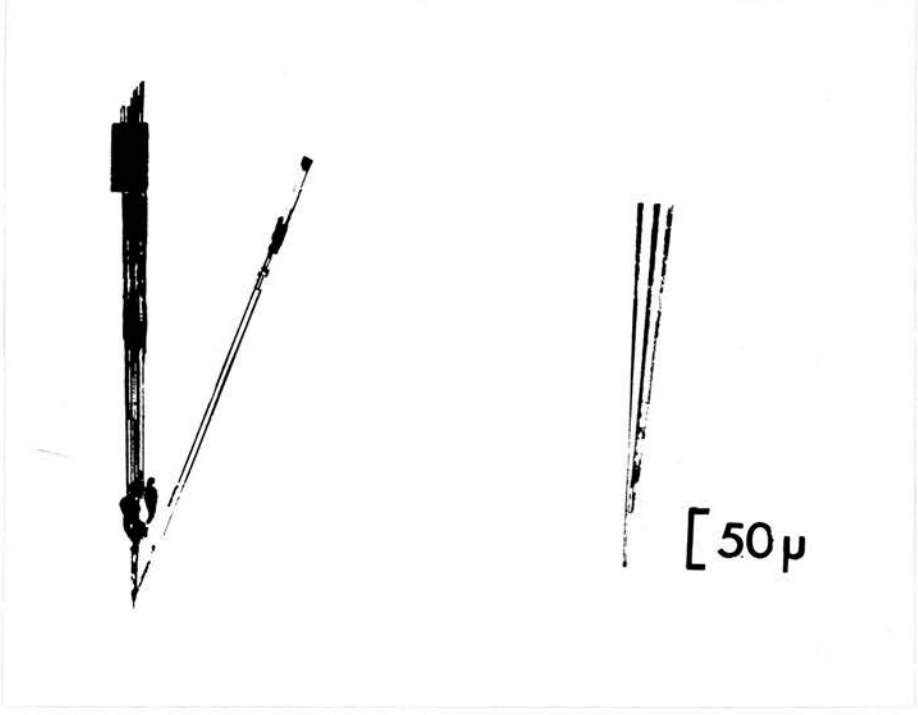

FIGURE 6

Photographs of parallel microelectrode. The photograph on the left illustrates the whole assembly consisting of iontophoresis barrel and recording electrode. On the right is shown a photomicrograph of the tip of the assembly. The single recording electrode projects about 40 μ beyond the iontophoresis barrels.

connected with the properties of the adhesive used to glue the electrode together. A successful adhesive must be non-toxic, quick-drying with little shrinkage and easy to apply, i.e. fairly fluid. Six adhesives were tested during the development of the electrode, the most successful was found to be Polystyrene Q-Dope (GC No. 37-2 Walsco No. 57-02W). Compound electrodes were constructed using one, two or five barrel iontophoresis electrodes. Single barrel recording electrodes were slightly modified for inclusion in a compound electrode, by lengthening the electrode shank to about 15 mm. Using a micro-forge (De Fonbrune), the recording electrodes were bent about 5 mm above the tip to an angle of about 40°C. Both the recording electrode and the iontophoresis barrel were then filled in the manner described above. Having broken back the electrode tips, the intracellular electrode was then mounted in a micromanipulator (Prior). The extracellular iontophoresis unit was attached to the stage of a binocular microscope. The two electrode tips were positioned precisely so that the recording electrode lay parallel and adjacent to the iontophoresis barrels. The recording electrode was adjusted so that it projected beyond the iontophoresis barrels by 30-70 μ. It became evident during this process of alignement that a uniformly tapered recording electrode was essential if the iontophoresis barrels were to lie immediately alongside. This close alignement is necessary if the two electrodes were not to be wedged apart as they passed through the tissue of the nervous system. A heavy wooden base was made upon which both the microscope and the micromanipulator were placed, this

common base reduced the possibility of any independent movement of the two electrodes. Small movements transmitted through the structure of the building (a result of the functioning of the lift) were occasionally troublesome.

When the two electrodes were aligned, the adhesive Polystyrene Q-Dope was slightly thinned using the appropriate agent (Polystyrene Q-Dope Thinner GC No. 41-2 Walsco No. 202-02). A blob of adhesive on the tip of a fine paint brush was drawn through the electrode tip and along the shanks. The adhesive invariably receded from the tip and there was never any problem of its blocking the tip. After about 10 minutes the adhesive was dry and a further two coats were applied.

Further stabilisation was provided by the use of a dental wax (Deiberit 502). The wax (molten when hot) was gently dropped between the upper parts of the two shanks. On occasions this procedure produced a lateral separation of the electrode tips (probably due to unequal shrinkage of the wax), thus every electrode was very carefully examined under the microscope before use. This examination included the application of lateral pressure to the electrode, if both tips bent as a unit without any lateral separation, it was assumed that the electrode was adequate for at least one track through the nervous system. Reliable interpretation of recorded effects requires that the drug be applied as near as possible to the cell from which intracellular recordings were being made. Thus it is extremely important that the iontophoresis and recording electrode remain in contact at the tip during drug studies. After each track the microelectrode assembly was microscopically

examined and rejected if there was the slightest indication of tip separation. It is possible that the downward movement through nervous tissue on occasions caused a separation of the tip, which was reversed when the electrode was retracted. However consideration of the capacitative coupling between the recording and iontophoresis electrode gave an indication of any possible tip separation.

Iontophoresis - Preparation and release of drug solutions

5-hydroxytryptamine

The bimaleate salt of 5-hydroxytryptamine (Sandoz) was used to fill an outer barrel of the microelectrode. The salt, at a concentration of 0.2 Molar, had a pH of 3.5. If the pipette is made positive with respect to the external solution, the resulting cationic current will carry the positively charged 5-HT moiety plus hydrogen ions out of the barrel since the salt probably ionises thus:

5-HT bimaleate $\quad 5\text{-HT}^{+} + H^{+} + \text{Maleate}^{=}$

Evidence that the maleate ion does not influence the responses of neurones to 5-HT is provided by a series of control experiments undertaken by Roberts and Straughan (1967) which have since been confirmed (Bradley, 1968). During the release of 5-HT, current balancing was achieved by applying a current of similar magnitude but opposite polarity from a sodium chloride containing pipette. A negative retaining current of 25nA was applied to the 5-HT-containing barrel when not in use.

Glutamate

A 0.5 Molar solution of sodium L-glutamate was used. The pH of the solution was adjusted to 8.5 using sodium

hydroxide. Glutamate ions are negatively charged and are thus ejected by a negative or anionic current. A positive retaining current of 25 nA was applied.

Cinanserin

Cinanserin (2' - /3-dimethylaminopropylthio/ cinnamalide hydrochloride) is a Squibb (SQ10643) product. The drug has been reported as having specific 5-HT antagonist properties in the periphery (Rubin, et al., 1964) and in the cat cerebral cortex (Roberts and Straughan, 1967). Cinanserin was iontophoretically applied in the form of a 0.1 Molar solution. The pH of the solution was adjusted to 3.0.

Lysergic Acid Diethylamide

The hydrochloride salt of LSD 25 was used. Since the substance is extremely costly and potent, only 0.5 ml. of 0.01 Molar solution was made up at any one time. The pH of the solution was 4.5. During retention a 25nA negative retaining current was applied to the barrel.

Stability of drug solutions

When not in use microelectrodes were always stored in a refrigerator (5°C). Although most drug solutions used in the microelectrodes are chemically stable over a period of at least one week, LSD is relatively unstable (M. Roberts personal communication). Thus microelectrodes containing LSD were only used for up to 3 days after being filled.

Iontophoresis

The technique of microiontophoresis (or microelectrophoresis) as applied to the central nervous system essentially involves the electrical ejection of potentially pharmacologically

active ions from fine glass tubes (microelectrodes or micropipettes) into the extraneuronal space. The electrical response of neurones affected by such ions is monitored by recording the extra- or intracellular potentials.

The recording of extracellular potentials is usually achieved with a sodium chloride- containing centre barrel of a multibarrelled electrode. The pharmacological activity of the ejected drug is assessed by noting the change in firing rate of the neurone. Since many neurones within the central nervous system are quiescent under experimental conditions, some workers have artificially induced a firing level by ejecting a small amount of an excitant substance (e.g. glutamate) from another barrel of the microelectrode. Another technique, one which has been used in the reported experiments, involves the iontophoretic application of agents whilst recording an evoked field potential. A field potential is usually regarded as being a reflection of the spike activity of a 'pool' of cells. An increase in the amplitude of the field potential may be interpreted as an increase in the number of cells producing action potentials.

Drugs which dissolve and become ionised in water are capable of being iontophoretically ejected. The iontophoretic application of a substance is accomplished by developing a potential gradient between the drug solution and the extracellular fluid. Anions i.e. ions bearing a negative charge will be ejected if the drug solution is made negative with respect to the extracellular fluid.

Although one of the basic laws of physical chemistry

states that the rate of ion transport of a fully ionised substance is proportional to the product of the current and the transport number of the ions, the relationship is far more complex when applied to the microiontophoretic situation. Since the transport number (a measure of ion mobility) varies with the ionic concentration of the drug both in and immediately around the tip of the electrode, it is difficult to calculate the exact quantity of released drug. However, using radioactively labelled drugs several groups of workers have obtained values for the transport numbers of:- acetylcholine, noradrenaline, 5-HT, LSD etc. (Krnjevic, et al., 1963; Bradley and Candy, 1970). These reports suggest that the release of such drugs is directly proportional to the charge (the product of time and current) passed when the tip diameters of the electrodes are comparable. There is however considerable variation in the calculated transport number between "different" microelectrodes basically the larger the tip diameter, the higher the transport number.

The variation in the iontophoretic release of poorly ionised substances such as LSD 25, is high. A contributory factor in this variation is the flow due to electrosmosis. Normally electrosmosis, the movement of ions within the bulk flow of solvent, contributes little to the efflux of substances from microelectrodes. However since the electrosmotic contribution of a poorly ionised insoluble substance to the total ionic flux is proportionally greater than that of a highly ionised substance, in the former case the electrosmotic effect may well be significant.

In addition to the applied electrical forces, ions within the microelectrode experience several other influences. Briefly, forces which tend to retain the contents of the microelectrode tend to dilute the drug solution at the tip of the electrode. Such forces are insufficient to prevent the net efflux of ions due to hydrostatic and diffusional forces. To balance the net efflux it is necessary to apply a retaining (backing) current to the electrode. The ionic concentration at the tip of the electrode probably varies from a maximum immediately after drug ejection to a much lower level when the 'resting' diffusion level has been reached. Consequently it is theoretically impossible to set the retaining current at a level at which it will just prevent the diffusion of ions from the microelectrode. Dilution of the tip concentration is of significance when comparing quantitatively drug effects on neurones. Recent reports (Bradshaw, et al., 1972) have confirmed experimentally these theoretical considerations.

The microiontophoretic technique enables extremely small quantities of substances to be locally applied to neurones in the CNS. Although relative proportions of released drug can be calculated from a knowledge of the quantity of current passed, the concentration of the drug at the target site can never be accurately assessed.

Occasionally, especially when the iontophoresis barrels are very close to a neurone, the extremely small currents (5-100 nA) used to eject ions, may themselves be responsible for changing the excitability of neurones. These current

effects may be annulled by applying a similar but opposite current from a sodium chloride containing barrel in the microelectrode. It is possible to distinguish between drug and current effects by noting the time course of the effect; a drug effect invariably has a finite latency to onset and persists for some short period of time after the current is terminated. Current effects are relatively instantaneous. It may also be possible to apply the test substance from two different electrode barrels, in both the cationic and anionic forms.

Although current effects have been seen to affect both brain stem and cortical neurones, the effect seems to be minimal with spinal interneurones and Renshaw cells (Curtis, 1964; Curtis and Koizumi, 1961).

It is well known that neurones are particularly sensistive to changes in the pH of the surrounding extracellular fluid. Since many drugs have to be dissolved in either an acidic or alkaline solution, note must be made of the possibility of a change in ionic form once the drug has reached the external medium. In addition hydrogen or hydroxyl ions will be passed as well as the drug.

There has been some controversy concerning the optimum pH of iontophoretically applied drug solutions. Recent reports (Frederickson et al., 197 , Jordan et al, 1972) claim that noradrenaline and 5-HT solutions at different pHs are capable of eliciting different responses from spontaneously active cells in the cat cortex viz - low pH solutions tend to excite cells whereas solutions at higher pH tend to depress

cellular activity.

These observations are however in direct contrast with those of Johnson, et al., (1969) who reported predominantly excitant actions of noradrenaline on cortical cells when applied from all solutions in a pH range 3.0 to 5.5. Earlier reports (Curtis et al., 1959, Curtis and Watkins 1960) tended to confirm these findings. Furthermore as has been recently indicated (Roberts, et al., 1972) it is difficult to explain how a range of α and β noradrenergic receptor antagonist can selectively and reversibly prevent noradrenaline excitations whilst not influencing depressant responses if these responses were a direct result of the release of hydrogen ions.

Practical Application

The reported experiments involving iontophoresis have involved the application of drugs close to motoneurones in the lumbar spinal cord. It has not been possible to apply the technique in the conventional manner i.e. observing the effect of iontophoretically applied drugs on spontaneous activity, since in the anaesthetised animal motoneurones do not fire spontaneously.

However the effect of various drugs has been observed on the synaptically evoked activity of motoneurones. Stimulation of either a dorsal root or a bulbo-spinal pathway may result in changes in the excitability of motoneurones. These changes in membrane potential have been monitored using both intracellular and extracellular recording techniques. The modification of these potentials by iontophoretically

applied drugs has provided information concerning neurotransmission at the motoneurone.

Photography

Field potentials recorded from the ventral horn of the lumbar cord together with ventral root potentials have been displayed on an oscilloscope. Traces were photographed via a Bioelectric Reflexor unit using a Grass C4 camera. Two types of Ilford film have been used during the investigation; an unperforated orthochromatic film 5G91 and a perforated panchromatic FP4 film. After being developed, the film was projected and the relevant measurements made. Identification of individual frames was straightforward since the Reflexor unit projected a different number onto the oscilloscope face for each photographed frame.

Histology

The brains from all animals providing data were examined histologically. The brain was removed at the end of the experiment and stored in formol saline for one week. By referring to the stereotaxic atlas, the brain was 'blocked' and frozen. Serial sections (40 μ) were cut in the plane of the stimulating electrode and stained with thionin. The position of the electrode track was easily confirmed from the slides. Fig. 7 is a photograph of a section of cat brain stem at the level of the facial nerve. The original position of 4 of the 5 monopolar stimulating electrodes are indicated by the series of 4 small perforations. It can be seen that the centre stimulating electrode coincides with the midline of the brain stem.

FIGURE 7 Photograph of a section through the brain stem of cat illustrating the position of the tips of the three more medial of the 5 monopolar stimulating electrodes.

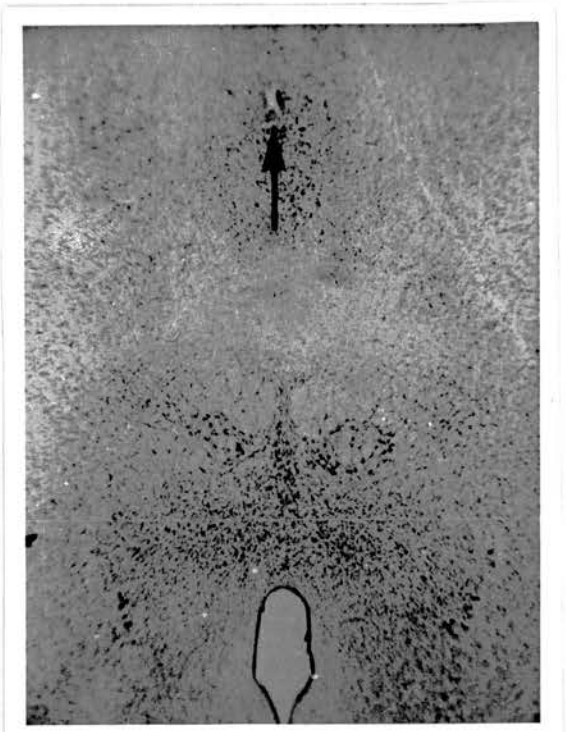

FIGURE 8 Photograph of section through brain stem of rat indicating the position of the stimulating electrode .

Fig. 8 illustrates the location of a single raphe nuclei stimulating electrode in the brain stem of rat at the level of trochlear nerve.

Electronic Circuits

The three different experimental procedures have each demanded a rather different arrangement of the electronic equipment.

Recording ventral root potentials via gross silver wire electrodes has involved the simplest electronic circuit. In these and all other experiments a Digitimer unit has been used to trigger both single impulses and pulse trains, the former applied to the dorsal roots, the latter to the brain stem. In addition, the Digitimer has been used to trigger the oscilloscope time base. A standard cycle period of 4 seconds has been adopted for all experiments, this rather long cycle period was chosen since it is important that activity evoked by stimulation of the afferent pathways has subsided before a second series of stimuli is applied (Lloyd 1943).

Electrical stimulation was provided by two Devices MKIV Isolated Stimulator units. One unit was used in conjunction with a Devices Gated Pulse Generator to provide pulse trains applied to the brain stem.

Biological activity was displayed on a Tektronix Type 502A dual-beam oscilloscope. In addition to photographing the scope face, the signal could be averaged using a data retrieval computer (Nuclear Chicago Model 7100). A permanent record of the computed response was obtained by photographing the oscilloscope display on the computer.

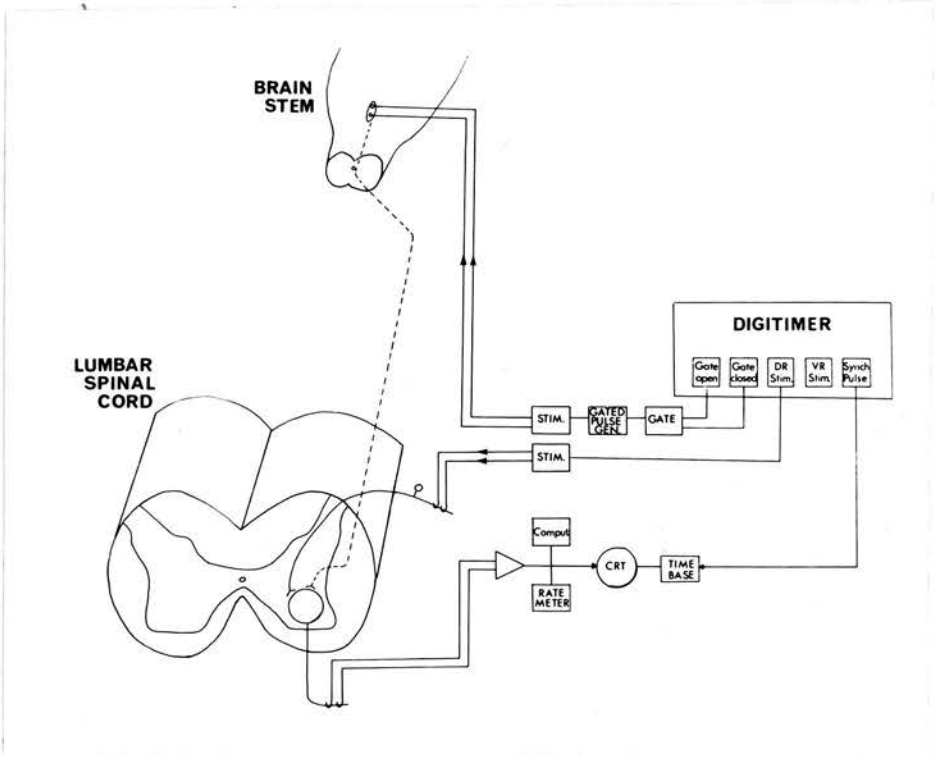

FIGURE 9

Basic electronic circuit for recording activity in the ventral roots.

The basic layout of the equipment is shown in Fig. 9.

The use of microelectrodes necessarily involves a much more complex recording circuit. The electrical characteristics of the microelectrode require a special adaptation of the input amplifying circuit to which the microelectrode is connected. The need for the adaptation arises from the mismatching of the oscilloscope amplifier input resistance (about $10^5\Omega$ - $10^6\Omega$) with the microelectrode resistance (about $10^7\Omega$). For the signal to be adequately amplified without any increase in the time constant of the input circuit, or any loss in signal, the oscilloscope amplifier input resistance should be considerably greater than the microelectrode resistance. The problem can be solved by using a high input resistance ($10^9\Omega$) electrometer placed between the microelectrode and the oscilloscope amplifier. The electrometer acts as an impedance transformer and has a low output impedance. The electrometer chosen for this series of experiments has been the Bioelectric NF1. This amplifier also has capacitance neutralisation and electrode resistance test facilities. A block diagram representing the arrangement of the electronic equipment used for microelectrode recording is shown in Fig. 10.

Experiments involving extracellular recording have invariably been combined with the iontophoretic release of drugs from multibarrelled micropipettes. The basic circuit for the iontophoresis unit, one channel of which is shown in Fig. 10, is based upon one developed in Professor Lundberg's laboratory (personal communication - M. H. T. Roberts).

abbreviations

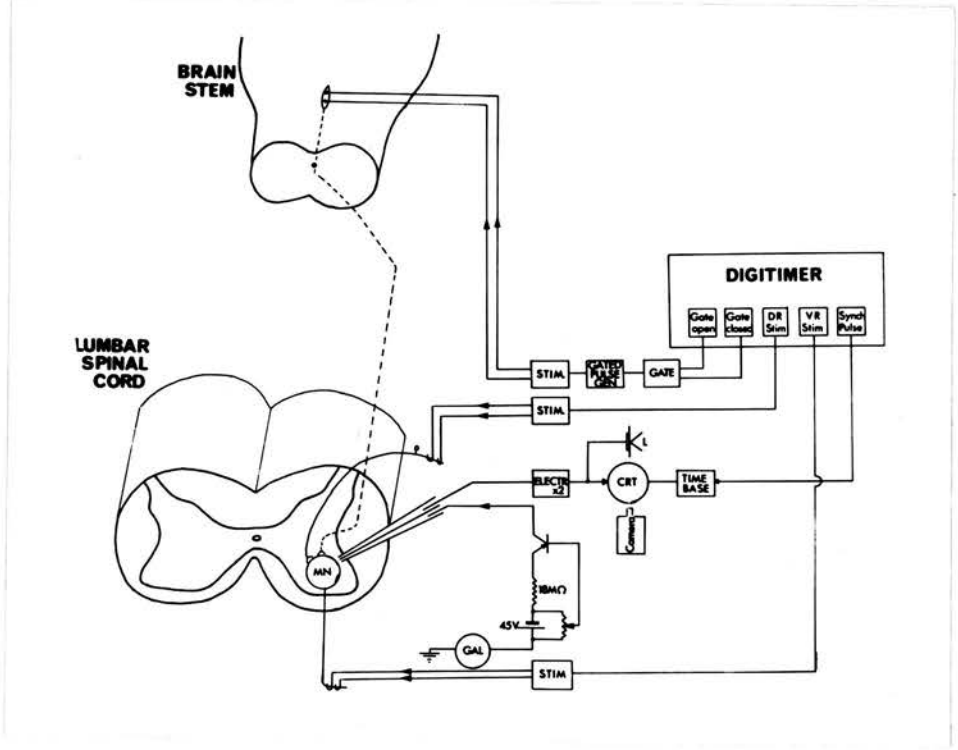

FIGURE 10

Basic electronic circuit for extracellular recording and iontophoresis.

The effective output impedance of the iontophoresis panel is greater than $10^{12}\Omega$, thus even a considerable increase in electrode resistance will remain insignificantly small when compared to the output resistance. As a result, the supplied current remains constant. Electrical pick-up is minimised by locating the shielded transistors close to the microelectrode.

More complex circuits were involved in the intracellular recording study. By incorporating the recording micro-electrode in one arm of a Wheatstone bridge circuit, it is possible to directly stimulate the impaled cell through the same electrode used for recording the intracellular potential. This technique, developed by Araki and Otani (1955) is based upon the four-arm Wheatstone bridge. Fig. 11 shows the circuit diagram. In addition to the basic circuit, a d.c. compensating unit has been included. This allows any standing d.c. potential to be annulled.

The calibration unit (Bioelectric Calibrator CA5.431) provides a pulse of known duration and amplitude which is displayed on the cathode ray tube (CRT) at a convenient point.

A Nuclear-Chicago Constant-Current Stimulator (Model 7150) has been used to provide a current source for pulses applied across the motoneurone membrane. Current pulses, triggered by the Digitimer were normally injected down the electrode when the electrode was extraceullular. The bridge was then balanced by adjusting the variable resistance in one of the bridge arms. Fig. 12 shows the relative position of the Wheatstone bridge circuit within the intracellular

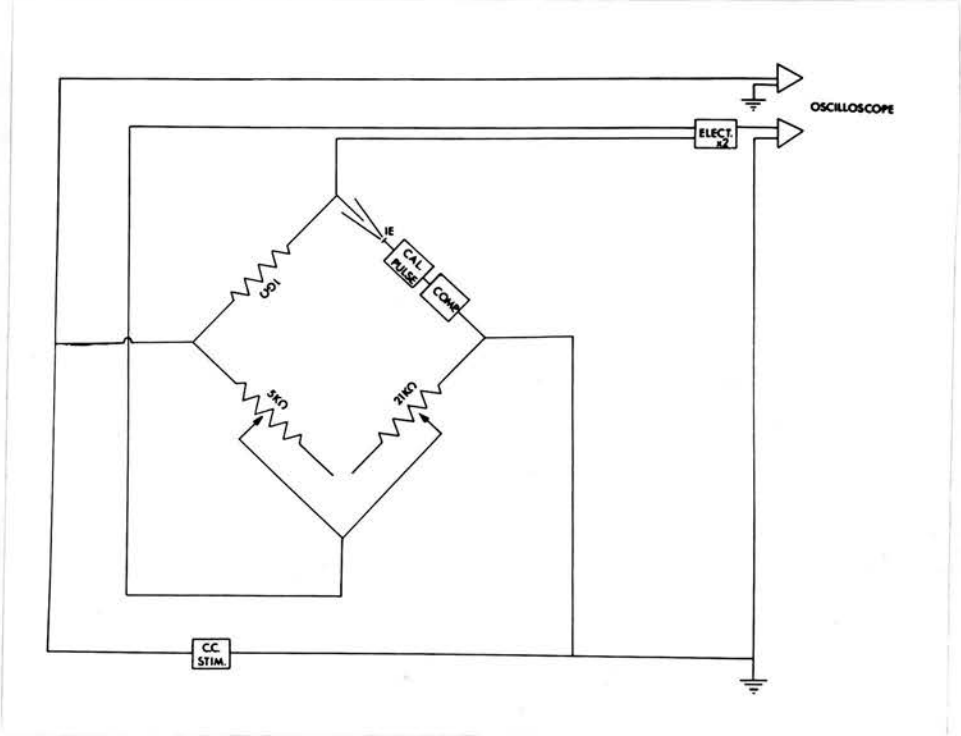

FIGURE 11

Wheatstone bridge circuit employed during intracellular experiments.

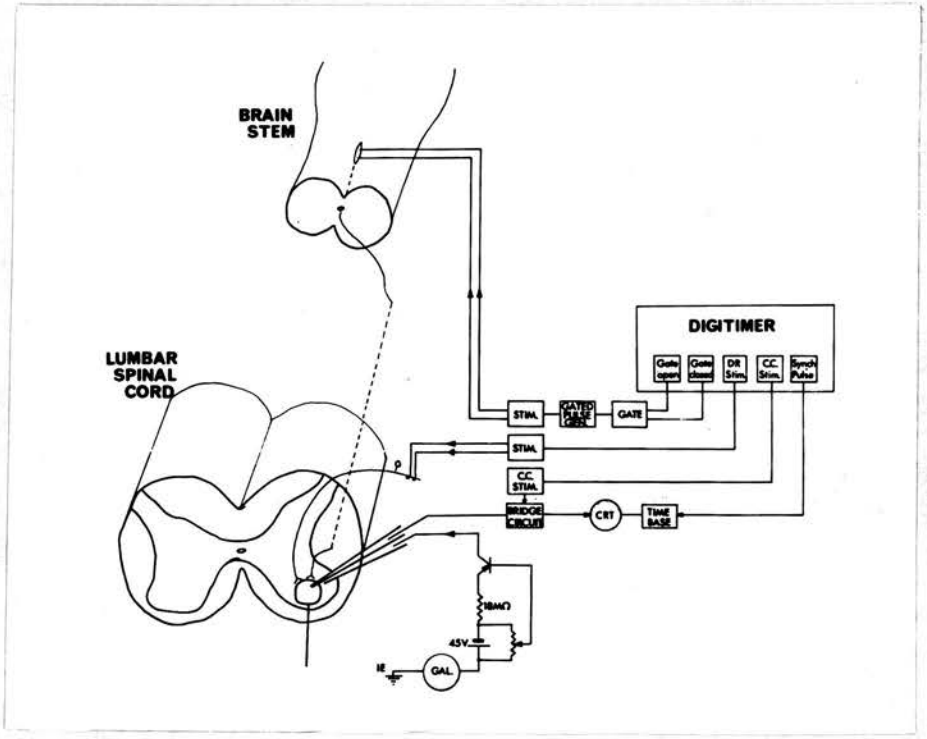

FIGURE 12

Basic electronic circuit for intracellular recording and iontophoresis.

recording circuit.

Experimental Design

Three different types of experiment have been performed during the reported project. Essentially the variations in experimental design have reflected the necessity to monitor the activity of a bulbo-spinal pathway. Since there is evidence to suggest that the relevant pathway terminates on lumbar motoneurones, it seems reasonable to postulate that activity in the pathway may result in changes of excitability of spinal motoneurones.

Excitability changes of neurones are manifested by changes in the membrane potential. Thus the most direct approach to measuring the excitability changes of neurones involves recording the membrane potential. This may be achieved using intracellular recording electrodes.

Electrical stimulation of the raphe-spinal pathway has been effected by stereotaxically placing a stimulating electrode in the raphe nuclei. It cannot however by assumed that all responses to stimulation are a direct result of the release of 5-HT. The contribution of the 5-HT pathway to the observed effects can be assessed using pharmacological antagonists. Thus, a range of 5-HT antagonists have been used to identify the extent to which 5-HT is involved in the reported modification of motoneurone excitability.

Experimental Design

-I- Intracellular recording

Experimental animal - cat and rat.

a) Brain Stem Raphe Stimulation (Conditioning Stimulation)

i) Varying the stimulation parameters.

ii) Comparing the effects of raphe and lateral reticular formation stimulation.

b) Identification of Post-Synaptic Response

i) Analysis of the intracellularly recorded post-synaptic response at the spinal motoneurone.

ii) Iontophoretic application of 5-HT antagonists to the motoneurone - effect on motoneurone response.

iii) Systemic administration of antagonist - effect on motoneurone response.

-II- Spinal Reflex Recording

Experimental animal - rat.

a) Brain Stem Stimulation (Conditioning Stimulation)

i) Stimulation of the raphe nuclei.

ii) Comparing the effects of raphe and lateral reticular formation stimulation.

b) Modification of Spinal Reflexes

i) Spinal reflexes evoked 20 msec. after the termination of the conditioning stimulation applied to the brain stem. The reflex was evoked by stimulation of dorsal roots.

ii) Systemic administration of 5-HT antagonists and precursors - the effect on the unconditioned (i.e. the reflex that is not preceded by brain stem stimulation) and the conditioned reflex.

-III- Motoneurone Field Potential Recording

Experimental animal - rat.

a) Modification of Extracellularly Recorded Field Potentials

i) Stimulation of dorsal and ventral root whilst recording in the vicinity of antidromically identified motoneurones.

ii) The effects of brain stem stimulation on the orthodromically evoked field potential in motoneurones.

iii) Modification of the unconditioned field potential by iontophoretically applied 5-HT.

iv) Modification of the conditioned field potential by iontophoretically applied 5-HT-antagonists.

R E S U L T S

Intracellular Recording

Aim

The aim of this study was to intracellularly record the post-synaptic responses of spinal motoneurones to electrical stimulation of the 5-HT-containing neurones in the raphe nuclei. Positive identification of a role for 5-HT in the mediation of the response was to be made by selectively blocking the intracellularly recorded response with specific 5-HT antagonists.

Recording from spinal motoneurones

A total of 331 intracellular recordings were obtained from spinal lumbar motoneurones. One hundred and ninety two recordings were provided by 40 cats, 139 cells being recorded in 101 rats.

Spinal motoneurones were antidromically activated by electrically stimulating the central end of the sectioned ventral root. Positive intracellular identification, was indicated when the stimulus-response latency was less than 0.5 msec., and by the response comprising of a single antidromic action potential. Occasionally intracellular recordings have been obtained from axons, primary sensory fibres and interneurones. However action potentials in fibres and axons differ from somato-dendritic spikes in not being accompanied by an inflection point in the rising phase, by their shorter duration and by the absence of a hyperpolarisation after the spike.

When recorded extracellularly, the firing of a neurone may be seen as an extracellular field potential. The con-

figuration of this field potential has been a useful guide in assessing the position of the recording microelectrode relative to the motoneurone.

Although no attempt has been made to histologically identify the position of the lumbar motoneurones studied, a note was made of the depth within the spinal cord at which each motoneurone was located. In cat, the motoneurone field commenced 2.0 mm and terminated 4.0 mm. beneath the dorsal surface of the spinal cord; in rat the field commenced at a depth of 1.2 mm. and terminated at about 2.0 mm. The optimum point of insertion for the microelectrode in the lumbar region of the spinal cord was found to be just lateral to the insertion of the dorsal roots with the microelectrode passing almost vertically through the spinal cord. At this position, two and often three motoneurones were recorded per track.

The successful penetration of a neurone was signalled by the recording of a membrane potential. Occasionally this was accompanied by an injury discharge and subsequent reduction in membrane potential. Investigation of such cells was not continued. Only those motoneurones with a recorded membrane potential greater than 50 mV and stable to within 5 mV were studied. On most occasions small transient fluctuations (synaptic noise) of the membrane potential were recorded.

Slight differences in the antidromically evoked action potential (or spike) were observed between cat and rat. For example, there was a distinct tendency for the after-hyper-

polarisation recorded from cat motoneurones to be greater than that recorded from rat motoneurones. In a sample of 32 rat motoneurones the mean after-hyperpolarisation was 3.4 mV, whilst the mean from a sample of 25 cat motoneurones was 6.9 mV. An example of the two different responses is illustrated in Fig. 13.

Similarly the average duration of the after-hyperpolarisation in rat motoneurones was 24 msec. whereas in cat motoneurones the figure was 38 msec. Qualitatively similar observations have been reported elsewhere (Bradley and Somjen, 1960). There was however no difference in the mean antidromic action potential amplitude between cat and rat motoneurones.

These variations may be explained either by differences in dendritic organisation or by the different degree of microelectrode damage suffered by cat and rat motoneurones (see Discussion). The first series of experiments involved stereotaxically locating a bipolar stimulating electrode in the nuclei of the raphe. The intracellular response of lumbar motoneurones to different types of stimulation has been investigated.

Intracellular responses to a single stimulus applied to the raphe nuclei

The majority of spinal motoneurones in both cat and rat did not respond to a single stimulus applied to the raphe nuclei. Both potassium chloride and potassium citrate recording electrodes have been used to record the predominantly

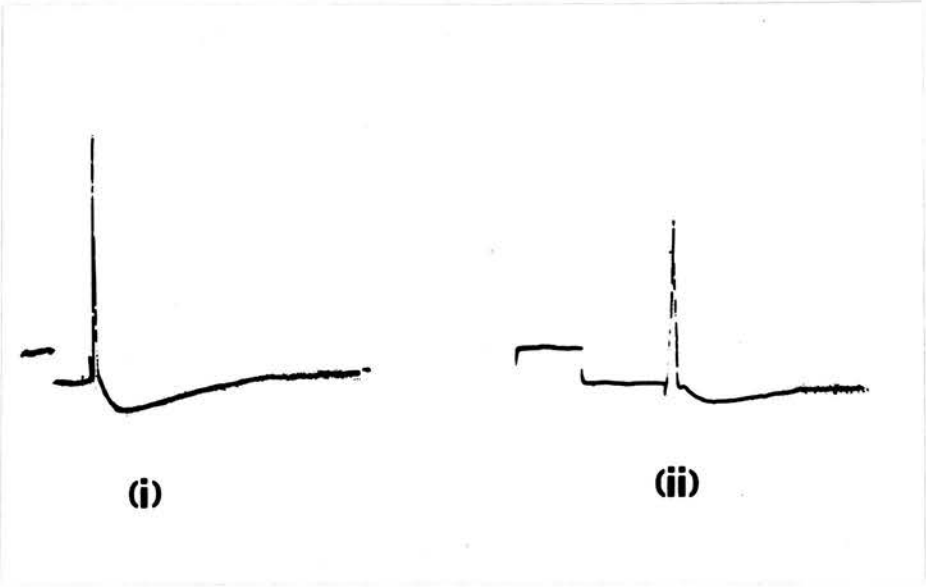

FIGURE 13

Comparison of after-hyperpolarisation in lumbar motoneurones associated with an action potential evoked by antidromic stimulation of ventral roots, in i) a cat motoneurone and ii) rat motoneurone. The calibration pulse represents 10 mV and 10 msecs. The vertical line immediately after the calibration pulse is the stimulus artefact.

excitatory response. When present, this response was recorded either as an EPSP or an action potential.

In rat lumbar motoneurones, the average latency of this EPSP was between 1.5 and 2.5 msec. In cat motoneurones the latency was between 2.5 and 3.5 msec. An example of an evoked EPSP in cat motoneurone is illustrated in Fig. 14 (i). The calibration pulse at the beginning of the trace is 10 mV in amplitude and 10 msec. in duration. The first stimulus artefact (a short vertical line transecting the base line of the trace) represents the point at which the ventral root was antidromically stimulated. The artefact is followed by the invasion of the motoneurone by the antidromic action potential. The second stimulus artefact indicates the time at which a single stimulus was applied to the raphe nuclei in the brain stem. The subsequent deflection of the trace represents the EPSP response of the motoneurone to the raphe stimulus.

After each intracellular study had been completed, the recording microelectrode was withdrawn from the cell and the raphe stimulation repeated. The absence of extracellularly recorded potentials indicates that the intracellularly recorded response cannot be attributed to local muscular activity resulting from brain stem stimulation. In addition, the fact that in 4 motoneurones a single stimulus evoked an action potential (see Fig. 14 (ii)) confirmed that the response was not due to muscle activity.

On several occasions in both cat and rat, in addition to an EPSP, a single stimulus as opposed to the more usual train of stimuli (see later), also evoked a slow depolarisation

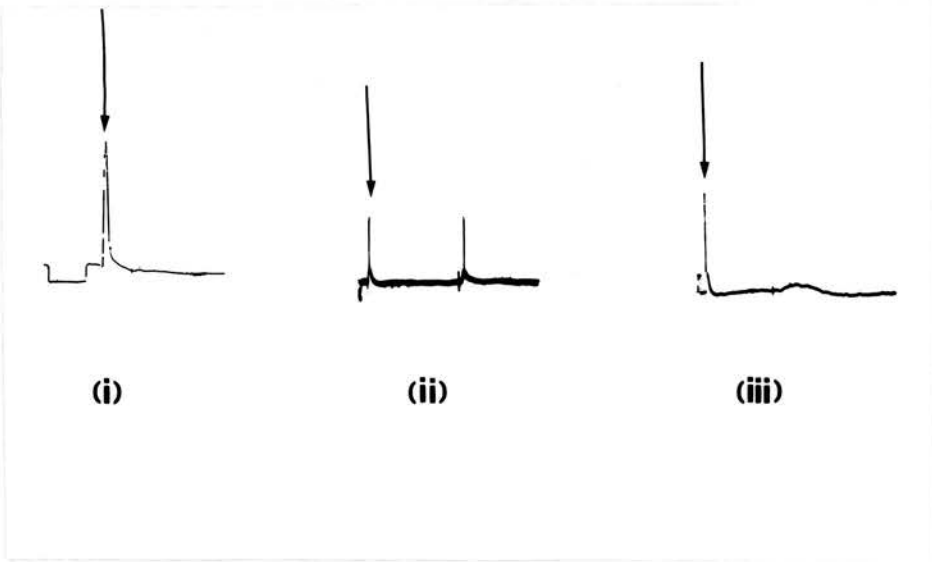

FIGURE 14

Intracellular responses of lumbar motoneurones to a single stimulus applied to the raphe nuclei. The responses were obtained from different motoneurones. Trace i) illustrates a short latency EPSP recorded from a cat motoneurone. The stimulus artefact is indicated by the vertical line. Trace ii) illustrates an action potential recorded from a cat motoneurone in response to a single stimulus applied to the raphe. Trace iii) illustrates an example of a slow depolarising response recorded from a cat motoneurone. Calibration pulse represents 10mV. and 10 msecs. The arrow indicates the position of the antidromically evoked action potential.

of the motoneurone. An example of this slow depolarisation recording from a motoneurone in rat is illustrated in Fig. 14 (iii). The slow depolarisation had a latency to onset of about 5 msec. and a duration of 25 msec. The maximum amplitude of this slow response was about 4 mV, a depolarisation which occasionally was itself sufficient to trigger an action potential. Unfortunately this response was not recorded with any regularity in either cat or rat and a detailed study was not possible.

Intracellular responses to a stimulus train applied to the raphe

Almost without exception, motoneurones responded to a 100 msec. train of stimuli at 100 Hz applied to the raphe nuclei. Although the response varied slightly from cell to cell and with different stimulus parameters, two components could be distinguished in most intracellular recordings.

After each individual stimulus in the train, there was a short latency, short duration (15-20 msec.) depolarisation. The time course and configuration of this response suggested it was an EPSP. The amplitude of the EPSPs often increased towards the end of the stimulus train (see Fig. 15 (i)). In addition, the EPSPs were almost invariably superimposed upon a slow membrane depolarisation. An example of this phenomenon is illustrated in Fig. 15 (ii). The slow depolarisation recorded from a cat motoneurone usually became evident after two stimulus pulses and increased at a steady rate throughout the stimulation period. The depolarisation then decayed from a maximum value coinciding with the end of the period of stimulation. Motoneurones in both cat and rat almost invariably responded in this manner.

A second component of the response to raphe stimulation

has been identified, again in both cat and rat motoneurones. Frequently the slow depolarisation associated with the EPSPs described above did not decay after the termination of the applied stimulation. In Fig. 15 (iii) the slow depolarisation actually increased in amplitude after the end of the stimulus train. The maximum depolarisation occurred 20 msec. after the last stimulus artefact. This recording is from a cat motoneurone whose membrane potential at this point was 60 mV. Occasionally the slow depolarisation was completely dissociated from the short latency responses, the maximum amplitude response being observed about 50 msec. after the end of the stimulus train (see Fig. 15 (iv)).

These more complex responses do not appear to be artefacts due to muscle activity since withdrawal of the recording electrode from the motoneurone always coincided with the loss of the response and administration of a muscle relaxant (under supervision) had no effect on the membrane responses.

Stimulation of lateral and medial regions of the brain stem

Having established the existence of a post synaptic response in lumbar motoneurones to brain stem stimulation, it seemed very important to determine whether these responses were selectively elicited from the region of the raphe nuclei. The comparatively larger diameter of the brain stem of cat suggested that this animal was more suitable for the study. The results are drawn from a group of 28 animals. A series of 5 monopolar tungsten stimulating electrodes, having an intertip distance of 2 mm., were arranged either in the coronal (20 animals) or sagittal plane (8 animals). Lumbar

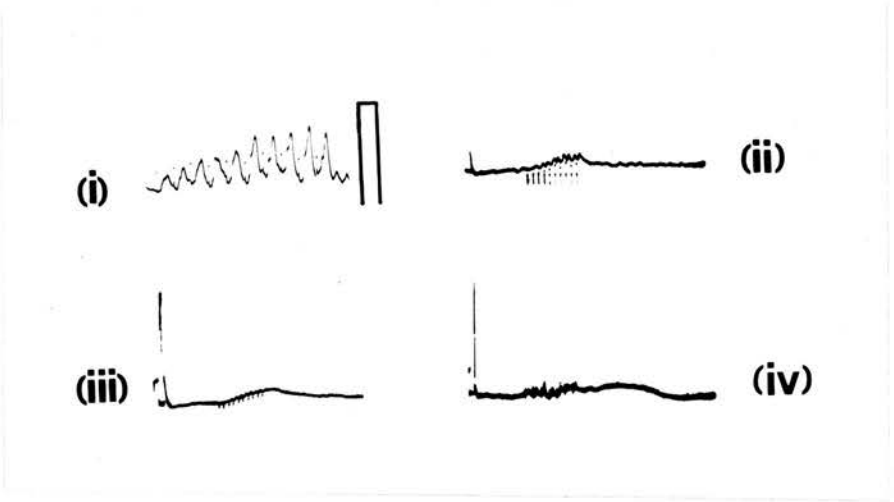

FIGURE 15

Intracellular rat / oat motoneurone responses to a 100 msec train of stimulus pulses applied to the raphe nuclei. Trace i) represents the short latency EPSPs. Trace ii) illustrates an example of EPSPs superimposed upon a slow membrane depolarisation. The depolarisation decays from the point of termination of the end of stimulation. Traces iii) and iv) illustrate both the short latency EPSPs and the slow membrane response, the latter being totally dissociated from the EPSPs in trace iv).

Calibration pulse represents 10 mV, and 10 msecs.

Calibration bar in trace (i) represents 10mV. and10msecs.

motoneurone responses to stimulation between adjacent pairs of electrodes have been examined in detail.

Stimulation in the coronal plane

In these experiments the 5 stimulating electrodes have been arranged across the brain stem. Before being introduced into the brain stem the stimulating electrodes were examined under the microscope to ensure that the amount of uninsulated tip (about 1 mm.) was similar in all the electrodes. In addition, the electrodes were electrically tested to determine the continuity of the insulation and resistance of the electrodes. Stimulating electrodes were only incorporated in the assembly if they conformed to a standard pattern (40-45 KΩ measured in NaCl by an Avometer). The centre electrode has been arranged to coincide with the midline. When located within nucleus raphe magnus, the central electrode was 2.5 mm. dorsal to the pyramidal tracts. Histological examination of the brain stem region of all animals studied has confirmed the positions of the stimulating electrodes. Although in a few animals there was some evidence of the misalignment of individual electrodes, the majority were very close to their target positions. Figures 7 and 8 illustrate typical examples of the histological determination of the location of stimulating electrodes in the brain stem of cat and rat respectively.

The region within the brain stem from which the short latency, EPSPs were elicited has not been entirely consistent. Although in some animals EPSPs were preferentially elicited from the 3 medial electrodes, in the majority of animals the more lateral structures were more sensitive to stimulation.

Thus activity in the pyramidal tracts is unlikely to be responsible for the postsynaptic effect. However the existence of a descending pathway originating from nucleus reticularis pontis caudalis, a nucleus lying about 1.5 mm lateral to the raphe nuclei, may account for the short latency EPSPs (see Discussion). By raising and lowering the stimulating electrodes it has been demonstrated that EPSPs are preferentially elicited at depths approximately corresponding to the raphe nuclei.

In contrast to the evoked EPSPs, the slow depolarisation has been clearly demonstrated only to be evoked from specific regions within the brain stem. In the 20 cats examined, the slow depolarisation was selectively elicited from the 3 most medial stimulating electrodes arranged across the midline. No evidence was obtained which might suggest that either the ipsilateral or contralateral electrode was more effective in eliciting the postsynaptic response.

In addition to identifying a medio-lateral specificity for response elicitation, the optimum depth within the brain stem from which the response is elicited has also been identified. The electrode assembly was moved through the vertical plane of the brain stem. Fig. 16 illustrates the amplitude of the slow response during stimulation between two of the three more medial stimulating electrodes at different depths in the brain stem. Zero on the abscissa corresponds to the stereotaxic position of nucleus raphe magnus. There is a small response when the electrodes are 1.5 mm. above the

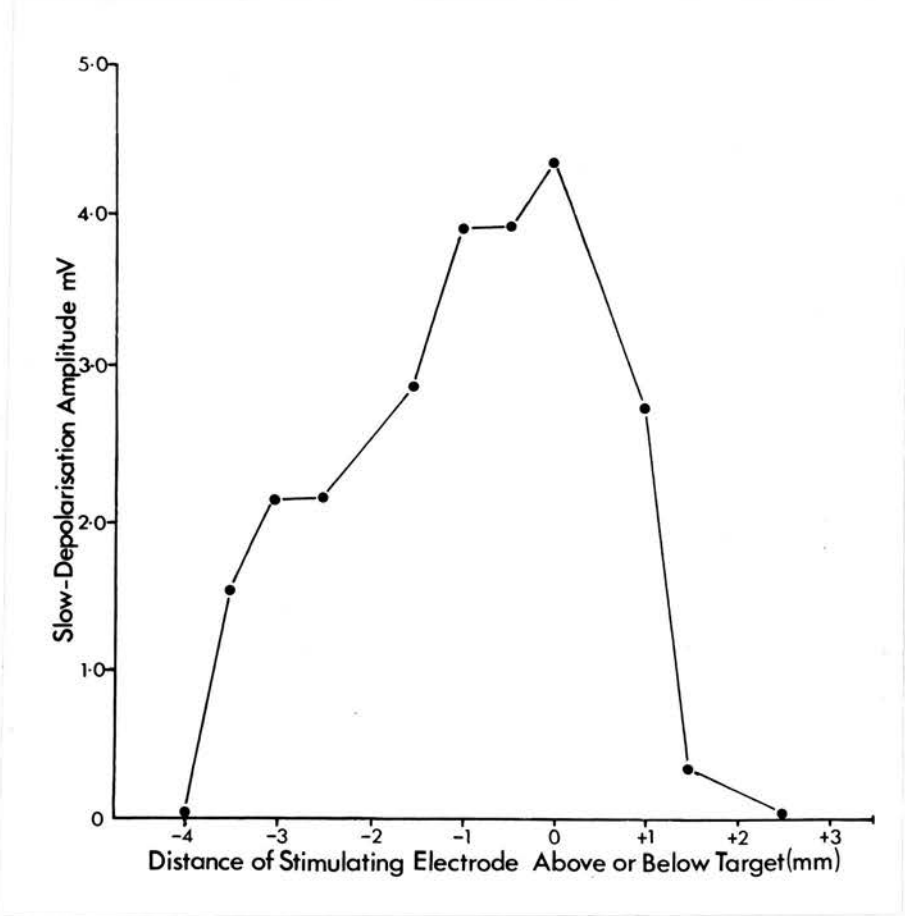

FIGURE 16

Relationship between the amplitude of the slow depolarising membrane response of cat lumbar motoneurones and the position in the brain stem from which the response was evoked. Stimulation between two monopolar electrodes in the midline in the brain stem. Target (0) is nucleus raphe medianus according to the co-ordinates of Snider & Niemer.

target, however the response amplitude increases markedly as the assembly is moved towards the ventral surface, and reaches a maximum at a depth corresponding to the nucleus raphe magnus. The response declines only gradually over the 2 mms. ventral to the target, possibly reflecting the diffuse nature of the nucleus.

Stimulation in the sagittal plane

In 8 cats the monopolar stimulating assembly was placed along the midline of the brain stem. The electrodes were arranged so that the middle electrode was located within nucleus raphe magnus. Since the tips of the individual electrode was 2 mm. apart, the more anterior electrode were outside the raphe complex. The arrangement of the electrodes within the brain stem is illustrated diagramatically in Fig. 2. As with the electrodes in the coronal plane, histological examination confirmed the positions of the electrodes.

Stimulation between successive pairs of electrodes has indicated that the EPSP type of post-synaptic response in motoneurones was apparently elicited from all regions of the raphe complex. Fig. 17 illustrates the amplitude and region from which these responses were elicited. The electrodes were moved through the brain stem in steps of 1 mm. The height of the bar in the diagram represents the average amplitude of the EPSPs evoked by a train of stimuli of constant intensity applied to that particular region of the brain stem. Although no response (the dot represents an absence of a response) was usually elicited from beneath the raphe nuclei, quite large responses were elicited both from within and above

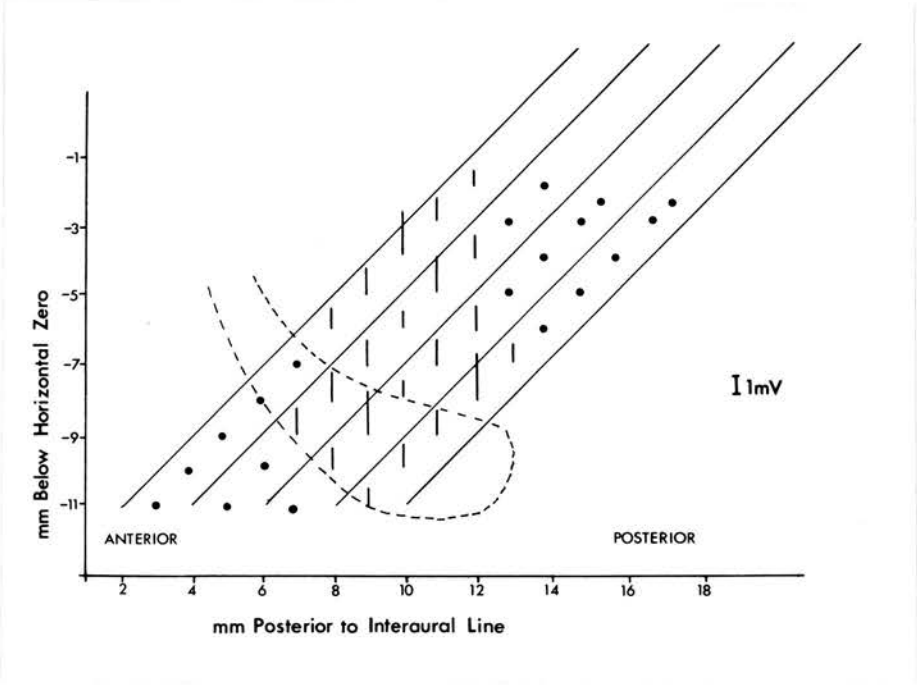

FIGURE 17

Diagrammatic representation of the amplitude of short latency EPSPs evoked from different regions along the midline of cat. The height of the bars along the electrode track represent the average amplitude of the EPSPs evoked at 1 mm. steps. The calibration mark represents a 1 mV response amplitude. The approximate outline of the raphe nuclei (derived from the stereotaxic atlas of Snider & Niemer 1963) is indicated by the dotted line superimposed on the figure.

the nuclei, Average maximum EPSP response was about 2.5 mV. Responses observed from stimulation of sites immediately above the limits of the raphe nuclei, the outline of which is marked in the figure by a dotted line, may be due to activation of descending fibres possibly originating from the more rostral nuclei. However it is apparent that the region of the brain stem from which the short latency EPSPs were elicited, did not coincide well with the reported location of the raphe nuclei.

Examination of the regions along the mid-line from which the slow depolarising response was evoked strongly indicates that the more posterior part of the nuclei are responsible for the response. Studies in 8 cats involving movement of the electrode assembly through the brain stem have confirmed that the origins of the slow response are either within or close to the posterior raphe nuclei.

Conduction velocity of the pathway mediating motoneurone responses

It has been established that the descending 5-HT-containing system in the spinal cord of rat consists of fine unmyelinated fibres having a diameter of between 1 and 2 μ (Dahlstrom and Fuxe, 1965). The only reported attempt (Couch 1970) to experimentally measure the conduction velocity in serotonergic fibres tentatively indicated a figure of between 0.6 - 0.8 m/s. This value seems reasonable in view of the fine nature of the pathway.

In contrast, the conduction velocity of the pathway mediating the short latency EPSP in response to stimulation

in the region of the raphe nuclei is considerably greater. In cat motoneurones the EPSP latency is between 2.5 and 3.5 msec. Assuming a pathway length of 15 - 25 cms, the upper and lower limits of the conduction velocity are 40 - 70 m/sec. A latency of 1.5 - 2.5 msec. has been recorded in rat motoneurone. Based on a pathway length of about 10 cms. the corresponding limits for the conduction velocity are 40 - 60 m/sec.

Based on conduction velocity measurements alone it seems unlikely that the short latency EPSP response in motoneurone results from activity in 5-HT-containing fibres.

The later slow depolarising response may however result from such activity. Based on the observation that the maximum amplitude of this response was recorded. in cat motoneurone about 50 msec. after the termination of raphe stimulation, the corresponding conduction velocity of the pathway is between 1 and 2 m/sec. (assuming a 15 - 25 cm pathway). This conforms well with the estimated conduction velocity of a 5-HT pathway. Although it is recognised that the above estimation of conduction velocities is approximate, the calculated conduction velocity indicated that the subsequent pharmacological investigation should be directed more towards the slow depolarising response.

Relationship between membrane potential and response amplitude

An interesting relationship has been identified between the level of membrane potential and amplitude of the evoked slow depolarisation. On several occasions the amplitude of the slow depolarisation in response to a constant stimulus

intensity has been monitored during the progressive loss of membrane potential. An example of this relationship is illustrated in Fig. 18. These results were obtained from a rat lumbar motoneurone. At a membrane potential of 70 mV, the slow depolarisation had an amplitude of about 6 mV, however 3 minutes later, after the membrane potential had been reduced by 10 mV, the response amplitude had been reduced by 25%. At 50 mV, the slow depolarisation had been reduced by about 60%. A similar relationship has been observed in 3 similar studies, one of which involved recording from a cat motoneurone.

The observation that changes in the membrane potential of less than 5 mV can significantly alter the response amplitude, indicated the dangers of comparing responses at slightly different membrane potentials. These observations were of direct relevance to the various antagonism studies in which the administration of antagonists was often accompanied by a change in membrane potential.

Current injection study

Artificially depolarising the resting membrane potential by between 5 and 30 mV. has been shown to substantially reduce the amplitude of the evoked EPSPs (Fig. 19 (i)). EPSPs whose amplitude was 2.5 mV at -70 mV membrane potential, were reduced to about 0.2 mV at a membrane potential of -35 mV. The slow depolarisations associated with the brain stimulation were also reduced by injections of depolarising current.

Conversely, the amplitude of the membrane response increased during periods of hyperpolarisation (Fig. 19 (ii)).

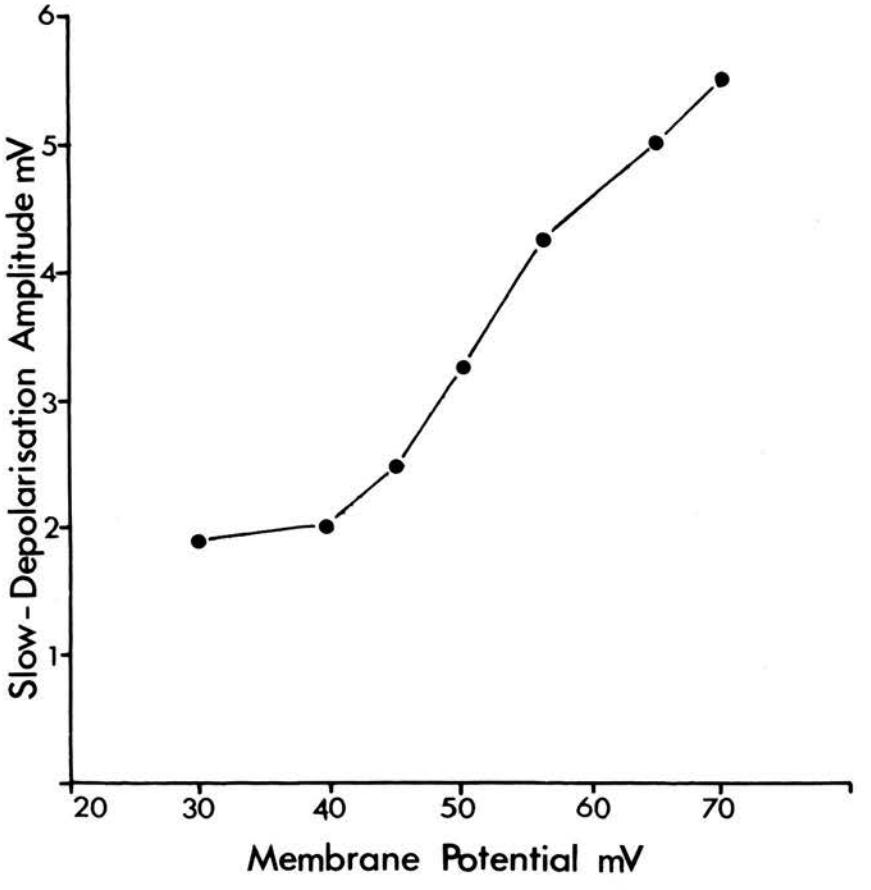

FIGURE 18

Relationship between membrane potential of (rat) lumbar motoneurone and amplitude of the slow depolarising response to raphe stimulation. The graph is constructed from the averaged responses of one motoneurone.

Artifically increasing the membrane potential by 20 mV doubled the EPSP amplitude and increased the amplitude of the slow depolarisation. Similar observations were obtained from 4 motoneurones studied. Care was taken to ensure that the Wheatstone bridge was balanced before intracellular recordings were made. However since membrane resistance measurements were not recorded during this study small imbalances were accepted.

Antagonism studies

The time course of the slow depolarising response, together with the fact that it was preferentially evoked from the region of the raphe nuclei, suggested that the response may have been due to activity in a monoaminergic pathway. It is recognised however, that activity in other descending pathways would also have accounted for the response (see Discussion). To determine whether any component of the motoneurone depolarisation was due to activity in the raphe-spinal pathway, 5-HT antagonists were applied to the immediate environment of motoneurones whilst monitoring the membrane responses.

Iontophoretic administration of antagonists

Originally it had been hoped to employ parallel microelectrodes to iontophoretically apply antagonists onto motoneurones whilst monitoring the membrane potential. Much time was spent on developing the technique of intracellular recording and extracellular iontophoresis and eventually a series of studies was completed using the technique. Fifteen cells

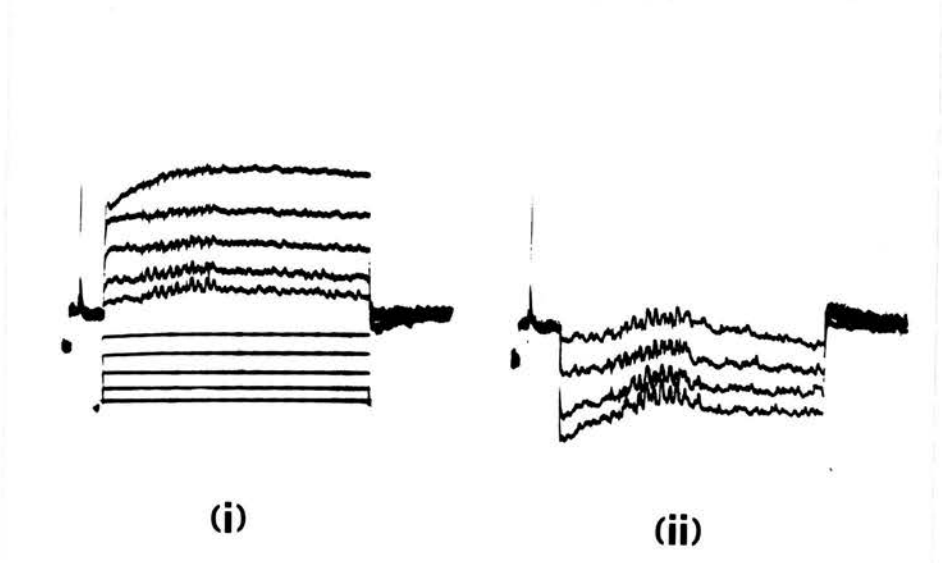

FIGURE 19

The effect of depolarising (i) and hyperpolarising (ii) current on motoneurone membrane responses to stimulation of the raphe nuclei. Calibration pulse: 10 mV and 10 msec.

were successfully studied in 9 cats. On 10 occasions cinanserin was applied, LSD was applied to the remainder. The latter drug was applied using currents of 25 nA for 3 minutes, cinanserin was applied for 3 minutes using currents of between 25-75 nA. Table 2 indicates the effect of antagonists on the membrane responses.

Antagonist	Number of Cells Studied	EPSPs		Long Duration Depolarisation	
		No Effect	Blocked	No Effect	Blocked
LSD	5	2	3	3	2
Cinanserin	10	7	3	6	4

Table 2

It can be seen from the table that the effects of the antagonists on the membrane responses are mixed. These equivocal results probably reflect the small changes in membrane potential associated with the technique. Previous iontophoretic studies (Roberts and Straughan, 1967, Bradley et al., 1971) have demonstrated the long time course of action of both LSD and cinanserin. It is suggested therefore that extremely stable membrane potentials over a period of at least ten minutes are essential if the effects of antagonists are to be observed. Very few studies were completed without any loss of membrane potential over the experimental period. In some cases the application of an iontophoretic current was immediately accompanied by a profound change in membrane potential. On such occasions the tips of the iontophoresis barrels were probably

intracellular or at least in contact with the neuronal membrane the change in membrane potential being due to the applied iontophoretic current. Fig. 20 is an illustration of the type of record frequently obtained during antagonism studies. Cinanserin was iontophoretically applied with a current of 25 nA for 2 minutes. After 100 seconds the slow response had become markedly reduced. Three minutes after the antagonist had been turned off, the response was no longer present. Although it seems likely that the loss in membrane potential could be directly responsible for the ultimate loss in response, during the application of the antagonist there was no such loss in membrane potential and yet the response declined markedly. The absence of recovery of the membrane response is not surprising in view of the later loss of membrane potential.

Systemic administration of antagonists

In view of the somewhat equivocal results obtained in the above experiments a more direct technique was applied. Membrane responses from motoneurones were recorded intracellularly using single barrelled electrodes, subsequently a 5-HT antagonist was injected systemically during the period of intracellular recording. On six occasions the antagonist was administered in this manner, and in a further four experiments the drug was given between intracellular recordings obtained from different motoneurones,

These ten successful experiments were drawn from a group of thirty similar experiments. The remaining twenty were not regarded as being satisfactory either because of unstable blood

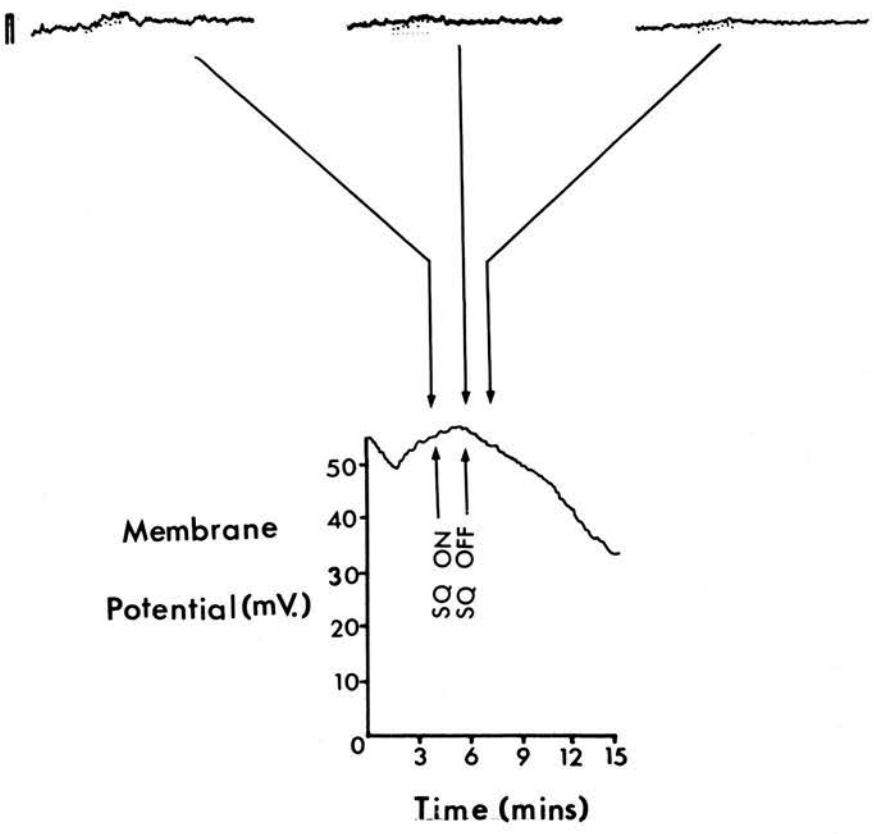

FIGURE 20

Effect of iontophoretically applied cinanserin (25 nA) on the slow depolarisation membrane response. Membrane responses evoked at different times before, during and after the application of the antagonist indicate some degree of attenuation of the response without a corresponding loss of membrane potential during the iontophoretic application.

Calibration pulse 10mV, 10 msecs.

pressures or problems associated with cardiac or respiratory pulsations. Cinanserin (2-6 mg/kg) was administered on three occasions, LSD (5-750 µg/kg) on seven.

There was little evidence to suggest that the antagonists at any dose were consistently blocking either the long depolarisation response or the short latency EPSPs. On two occasions in which the antagonist was applied, the short latency EPSP response was attenuated (see Fig.21). Thirty minutes after the antagonist had been applied in this cell there was some indication that the response had been attenuated. Careful examination of the membrane potential at this point however shows that about 8 mV of potential had been lost - probably sufficient to account for the reduced response amplitude. The effect of LSD upon the blood pressure, as illustrated in Fig.21, is rather unusual. On most occasions the slow systemic administration of the antagonist had little influence on the blood pressure. In fact care was taken not to cause any change in the blood pressure, since frequently such changes were associated with the loss of membrane potential during an intracellular recording.

The results of this intracellular study, together with those of the iontophoresis study were rather inconclusive. Two different types of response had been observed, a short latency EPSP and a longer duration depolarisation. From a theoretical viewpoint, the long depolarisation seemed more likely to have been associated with activity in a monoaminergic pathway. However results from both experimental techniques involving

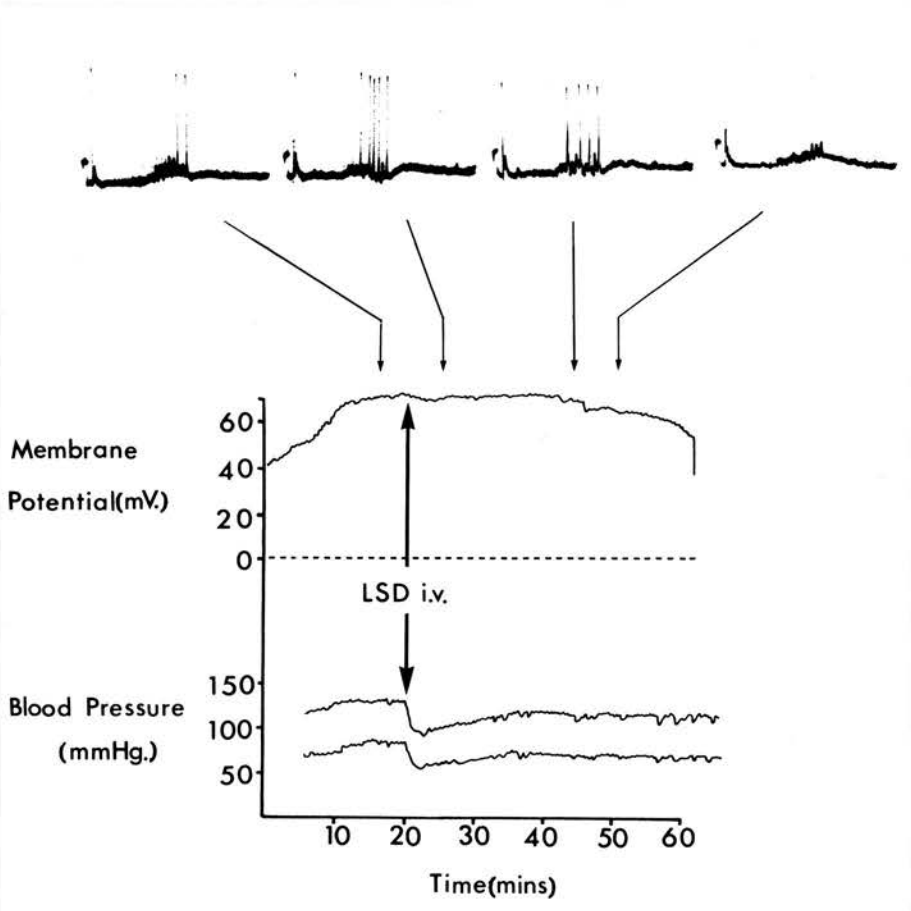

FIGURE 21

The effect of intravenous LSD (25 µg/kg) on membrane responses of a rat lumbar motoneurone. Before the drug was administered, stimulation of the raphe nuclei evoked several action potentials during the period of stimulation. After LSD, the rate of elicitation of spikes was markedly reduced. Note the gradual loss of membrane potential.

Calibration pulse for membrane responses 10 mV, 10 msecs.

application of antagonists did not confirm or deny a possible serotonergic basis for the response. Data from the histological examination of the location of stimulating electrodes suggested that the long duration motoneurone responses were elicited from a region of the brain stem corresponding to the nuclei raphe.

Based upon conduction velocity measurements and both physiological and pharmacological observations, the short latency EPSPs do not appear to result from activity in fine 5-HT-containing fibres.

II. Spinal Reflex Recording

On several occasions it was possible to record spontaneous activity in ventral roots emanating from the lumbar spinal cord of rat. This activity was recorded using two silver wire electrodes between 3 and 4mm. apart placed under a ventral root. The recorded activity represents action potentials from motoneurones conducted down the ventral root. The signal size as recorded from the ventral root was between 250-350μV, the non-biological component was about 100μV. Changes in the level of activity were assessed by a ratemeter, a permanent record being made using a polygraph.

Recording the spontaneous activity in the ventral root confirmed the profoundly depressant nature of higher levels of Fluothane. Since the number of action potentials arriving at the silver wire recording electrodes reflects the activity of the motoneurone pool, a reduction in the rate indicates a reduction in the excitability of the motoneurone pool.

It became evident that the levels of Fluothane required during surgery (1.0-1.5%) completely supressed spontaneous activity in ventral roots. By reducing the level to 0.5% for a period of at least 90 minutes, a reasonably stable level of activity was achieved on occasions.

At this lower level of applied Fluothane electrical stimulation applied to the raphe nuclei invariably increased the resting discharge recorded from the ventral roots. In these experiments brain stem stimulation consisted of ten successive 100 msec. trains of pulses applied at 1 sec. intervals. Fig.22 illustrates the effects of such stimulation. The upward deflection of the trace indicates an increase in the number of recorded action potentials, full scale deflection represented 100 spikes per second.

From the extract of polygraph record presented in Fig.22 it is clear that during the 10 second period of stimulation, activity within the ventral root was dramatically increased. Although part of the response is due to stimulus artefact, the major component is the biological signal. The post-stimulation period of about 2 seconds was characterised by a maintained level of activity in the ventral root. Subsequently the level of activity recovered to pre-stimulation levels over a period of between 60 and 80 seconds. In each particular experimental animal, the shape of the ratemeter trace was very similar after each period of stimulation.

However, 30 seconds after the intravenous injection of LSD (10µg/kg), the response recorded from the ventral

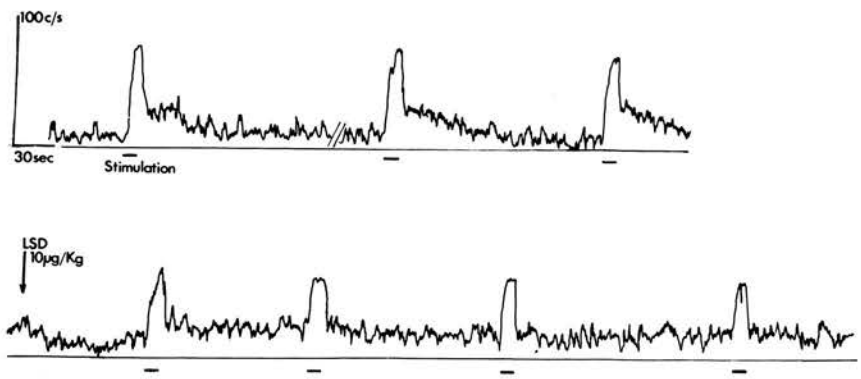

FIGURE 22

Extract of polygraph record illustrating the effect of intravenously applied LSD (10µg/kg) on the discharge recorded from ventral roots and evoked by stimulation of raphe nuclei in rat. The bar beneath the trace represents the period of stimulation. The increase in amplitude of the trace represents an increased motoneurone discharge. The effect of LSD is to almost abolish the prolonged discharge to stimulation of the raphe nuclei.

root to raphe stimulation had become much smaller. The response coinciding with the period of stimulation was reduced in amplitude by about 30%, however the prolonged 'after-discharge' was abolished. There was no indication of recovery 40 minutes after the antagonist had been applied. In three experiments the muscle relaxant Flaxedil (i.v.3mg/kg) was administered (under supervision) to the preparation. The relaxant did not noticeably affect the recorded level of activity. This would suggest that activity in the γ-loop system was not responsible for the recorded responses to raphe stimulation.

The six rats in which the activity within the ventral roots was monitored, all indicated that stimulation of the raphe nuclei can elicit an increase in the excitability of the lumbar motoneurone pool. Furthermore, it seems quite clear that LSD in small systemically applied doses profoundly modifies the raphe evoked discharge. However there are several reasons why the study of the spontaneous and evoked discharge in ventral roots was not pursued. Small, apparently spontaneous fluctuations in the level of excitability of the motoneurone pool invalidated the quantitative analysis of many preparations. Although the applied level of anaesthesia is constant, it may be that small fluctuations in the anaesthetic level, not normally significant, may interfere with the level of spontaneous motoneurone excitability.

To obtain a more precise estimate of changes in excitability of the motoneurone pool, the more traditional

method of recording the monosynaptic and polysynaptic reflexes evoked by dorsal root stimulation have been employed.

Ventral Root Reflex Recording

Ventral roots emanating from the lumbar spinal cord of rat were used to record reflex activity. The reflex was evoked by stimulating a dorsal root using two silver wire electrodes and recorded from the corresponding ventral root also via two silver wire electrodes.

Stimulation of the whole dorsal root produced a series of reflex potentials recorded from the ventral roots (see Fig.23). The first potential was invariably the largest and usually had a latency to peak of between 1 and 2 msec. This potential resulting from activity in a monosynaptic pathway had an amplitude of about 0.2mV., immediately after completion of the surgery. However, after a period of between 90 and 120 minutes, when the animal had become equilibrated at a lower level of anaesthesia, the monosynaptic reflex (MSR) was frequently 2 mV in amplitude.

A second more diffuse component of the ventral root reflex had a much longer (2-4 msec.) latency to onset. As can be seen in Fig.23, these later responses are easily differentiated from the earlier MSR. This later activity reflects activity in polysynaptic pathways (Lloyd 1943). The amplitude and duration of this activity varied markedly not only between one animal and another but also between neighbouring ventral roots in the same animal.

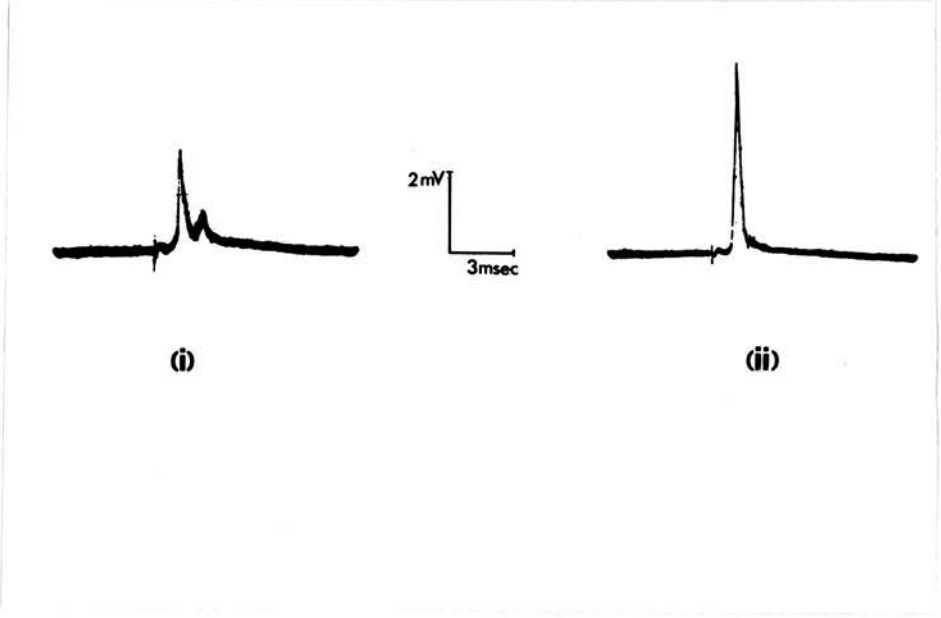

FIGURE 23

The effect of conditioning stimulation on the MSR recorded from the lumbar spinal cord. Trace i) represents three superimposed consecutively evoked MSRs, trace ii) represents a comparable reflex evoked immediately after conditioning stimulation. Note the increase in amplitude of the MSR and the absence of the late polysynaptic reflex (PSR) in the conditioned trace.

The majority of experiments have been primarily concerned with the quantitative analysis of the earlier monosynaptic reflex. Therefore several experiments were performed to assess the stability of the MSR over periods of between 3 and 5 hours. Although in the majority of animals the MSR remained constant for such periods of time, occasionally animals did not respond in a consistent manner to dorsal root stimulation. Preparations in which the MSR varied by more than 10% were not used.

Both the core (rectal) and paraffin bath temperatures have also been shown to profoundly affect both the amplitude and latency of the MSR. A fall in rectal temperature of 3° C from the normally maintained body temperature of 37°C, resulted in a prolonged latency to onset of the MSR. The latency was often increased by about 1 msec. Although the rectal and bath temperatures were not altered independently, it became evident that the bath temperature had to be maintained at 37 ± 1°C to achieve a replicable reflex. To ensure that small variations in the response were averaged , three successive superimposed traces were photographed from the oscilloscope face.

Initially an average transients computer was used to average 10 consecutive reflexes. Since a reflex was only evoked every 4 seconds, a series of computations often lasted several minutes severely limiting the experimental design. The computing facilities have however been sparingly used to confirm the consistency of the evoked reflexes.

Stimulus-Response curve

Stimulation of the dorsal root at increasing intensities produces an MSR of correspondingly greater magnitude. The relationship between MSR amplitude and dorsal root stimulation intensity is shown in Fig.24. The MSR amplitude increases in an almost linear fashion over a small range of dorsal root voltages. The maximum response is achieved at a dorsal root voltage of about 8 volts.

Both the dorsal root and the brain stem stimulating intensities have been expressed in terms of the applied voltage. Although it is more satisfactory to express the applied stimulation in terms of current strength, this has not been possible in the present study since the stimulating electrode resistance has not been continuously monitored throughout the experiments. However in the case of the raphe stimulating electrode, an initial measurement of the resistance was made at the beginning of each experiment. Where considerations of current spread have been discussed, stimulating currents based upon the initial resistance reading have been quoted.

The figure also illustrates the effect anaesthesia can have upon the reflex amplitude. The two curves were obtained from the same animal at two different levels of anaesthesia. The deeper level tends to slightly depress spinal reflexes evoked at lower dorsal root voltages and profoundly depress the maximum response. Constant response amplitudes were obtained over a period of one hour before any experimental procedures were initiated. Having once

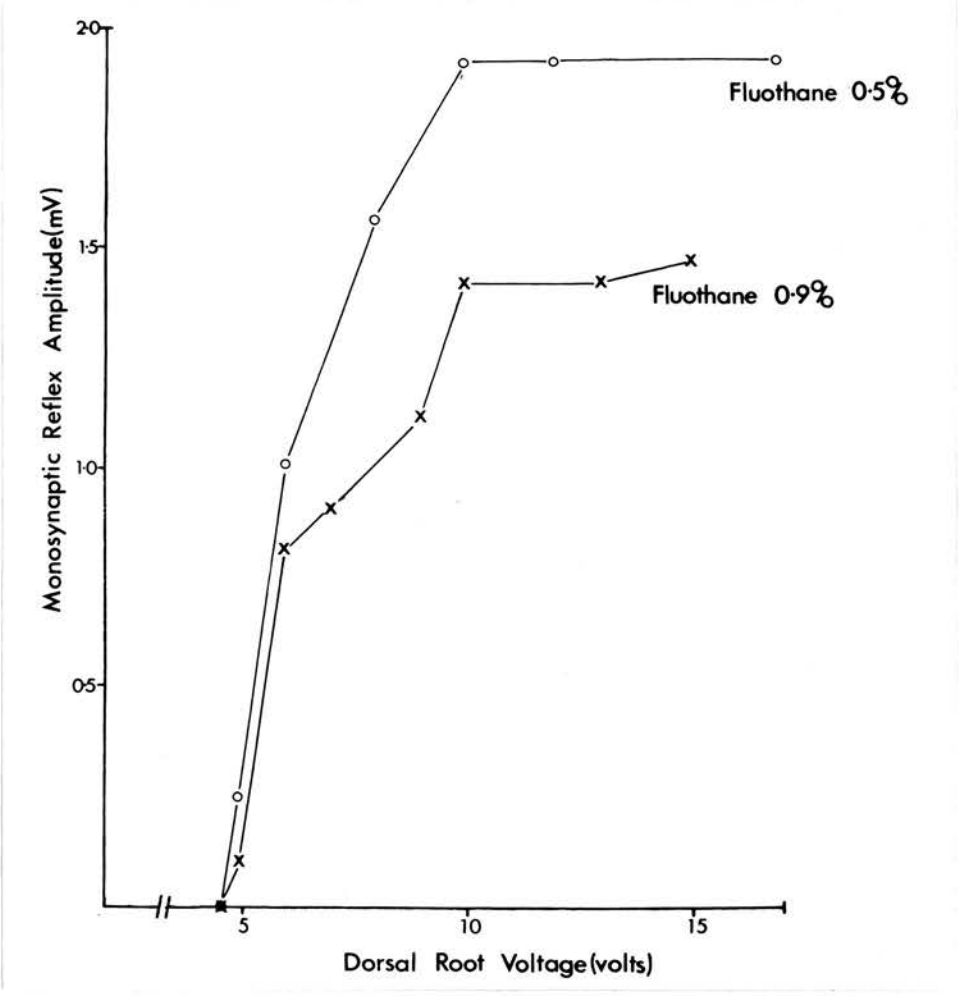

FIGURE 24

This graph illustrates the relationship between dorsal root stimulus intensity and MSR amplitude. The sensitivity of the 'stimulus-response curve' has been altered by changing the level of anaesthesia. Increasing the depth of anaesthesia reduced the amplitude of the MSR when compared to the MSR evoked at lower levels of anaesthesia. Each point represents the mean of three consecutively evoked reflexes. The graph was constructed from the results of one experiment involving a rat.

attained this uniform level of excitability, the MSR amplitude remained constant over a period of up to five hours.

Potentiation of the monosynaptic reflex

The amplitude of the MSR reflects the level of excitability of a sample of motoneurones from the lumbar motoneurone pool (Lloyd 1943). Changes in amplitude of the reflex correspond to changes in the number of motoneurones sending action potentials down the ventral root. The amplitude of the MSR is profoundly increased when it is evoked 20 msec. after the end of a stimulus train applied to the brain stem. The brain stem stimulation is thus regarded as a conditioning stimulation and will be referred to as such.

Emphasis has been laid on the potentiating effects of conditioning stimulation; this seems reasonable since the vast majority of animals responded in this manner. Occasionally however, conditioning stimulation was ineffective in modifying the amplitude of the MSR. On several occasions the absence of potentiating effects of conditioning stimulation was restricted to the earlier stage of the experiment i.e. immediately after the termination of the surgery when the animal was relatively deeply anaesthetised. This observation suggested the possible interference of relatively high levels of Fluothane with the descending effect. However, no firm relationship has been established between the level of anaesthesia and the presence or absence of the descending potentiating effect.

An example of the potentiating effect of conditioning stimulation on the MSR is illustrated in Fig.23. It can clearly be seen that the amplitude of the reflex evoked after the conditioning stimulation (i.e. the conditioned reflex) is considerably greater than the unconditioned reflex, (i.e. the reflex evoked in the absence of conditioning stimulation). In this typical example of the effect of conditioning stimulation, the conditioned reflex is 70% greater in amplitude than the unconditioned reflex. A second effect of conditioning stimulation was to significantly reduce both the latency to onset and the latency to peak of the MSR. In a group of 10 rats in which latencies were examined, the average reduction in the latency to peak response was 0.5 msec. Further effects of conditioning stimulation on spinal reflexes will be examined later.

The extent of the MSR potentiation has been observed to be dependent upon several variables. As would be expected changing the parameters of stimulation (both intensity and frequency) directly modifies the potentiating effect. In addition the conditioning-dorsal root stimulus interval, the dorsal root voltage and the exact position of the raphe stimulating electrode also affect the degree of MSR potentiation. These variables have been investigated in some detail.

Conditioning-dorsal root stimulus interval

The interval between the end of the conditioning stimulus train and the evoked MSR appeared to be important. Fig.25 represents the extent of potentiation at different

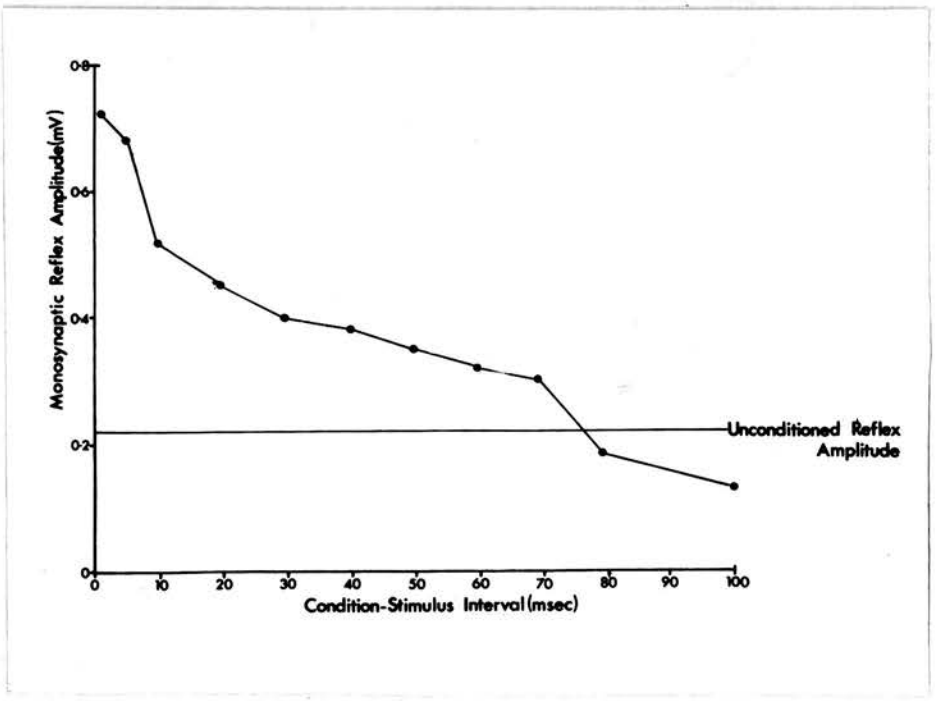

FIGURE 25

The relationship between conditioning-stimulus interval and extent of MSR potentiation. Each point represents the mean of three consecutively evoked reflexes, the results were obtained from one experimental rat.

condition-test intervals, the graph is constructed from readings obtained from one animal, however similar effects were observed on other occasions. Immediately after the termination of the conditioning stimulation the MSR potentiation seems particularly pronounced, the reflex being trebled in amplitude. Thirty msecs. after the condition train had terminated, the degree of potentiation was markedly reduced, and after 75 msecs. there was no effective recorded potentiation.

Position of raphe stimulating electrode

The exact location of the electrode tip has been determined in every rat from which experimental results were obtained. Of the 60 histologically examined brains, 85% indicated that the electrode tip was either within or not more than 1 mm. from the raphe nuclei. By comparing the degree of descending MSR potentiation in the animals in which the raphe electrode was placed some distance from the raphe nuclei it was hoped to correlate the degree of potentiation with the proximity to the raphe nuclei. In 2 rats in which, due to misreading of the stereotaxic equipment, the stimulating electrode was between 1.5 and 2.0 mm. from the raphe nuclei, substantially greater stimulus intensities were required to elicit the normally observed MSR potentiating effects. However, since electrode positions very close to the raphe sometimes appeared to be relatively insensitive to stimulation, little reliability is attached to the relationship between electrode position and MSR potentiation. It seems likely that there are other factors

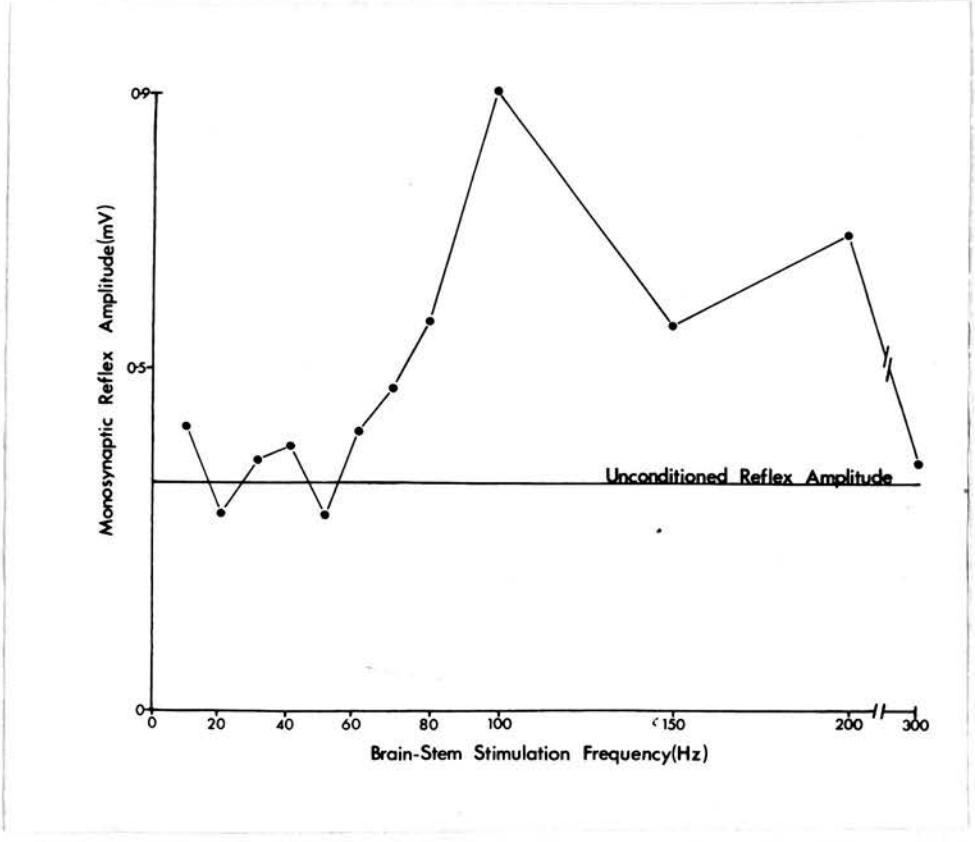

FIGURE 26

Influence of conditioning stimulating frequency on MSR amplitude. Each point represents the mean amplitude of three consecutively evoked reflexes.
The graph is constructed from the results of one experiment.

controlling the degree of MSR potentiation (see Discussion). Fig.8 is a photograph illustrating the position of a raphe stimulating electrode in a rat. The section is taken from the brain stem at the level of the IV cranial nucleus. The tip of the electrode is placed in the midline within the limits of the nucleus raphe medianus.

Stimulus parameters

By altering both the intensity and frequency of raphe stimulation it was possible to modify the extent of the descending influence. Fig.26 depicts the relationship between the stimulus frequency and MSR amplitude. The data is drawn from one rat. Stimulation frequencies below 60 Hz. apparently did not profoundly influence the response, the frequency corresponding to the greatest potentiation occurred at 100 Hz. and was thus adopted as the standard stimulation frequency. At frequencies greater than this the potentiation was markedly reduced. Possibly stimulation at frequencies above about 100 Hz. activates neighbouring descending pathways whose effects are predominantly inhibitory. Each point on the graph represents the average of three reflexes evoked at a constant interval of 4 seconds.

In most animals stimulating currents lower than about 0.06 mA. (at 100 Hz.) had no effect on the amplitude of the MSR. Between about 0.06-0.2 mA, the relationship was almost linear (see Fig.27). The chosen stimulating current varied from one animal to another but was usually within the range 0.08-0.14 mA.

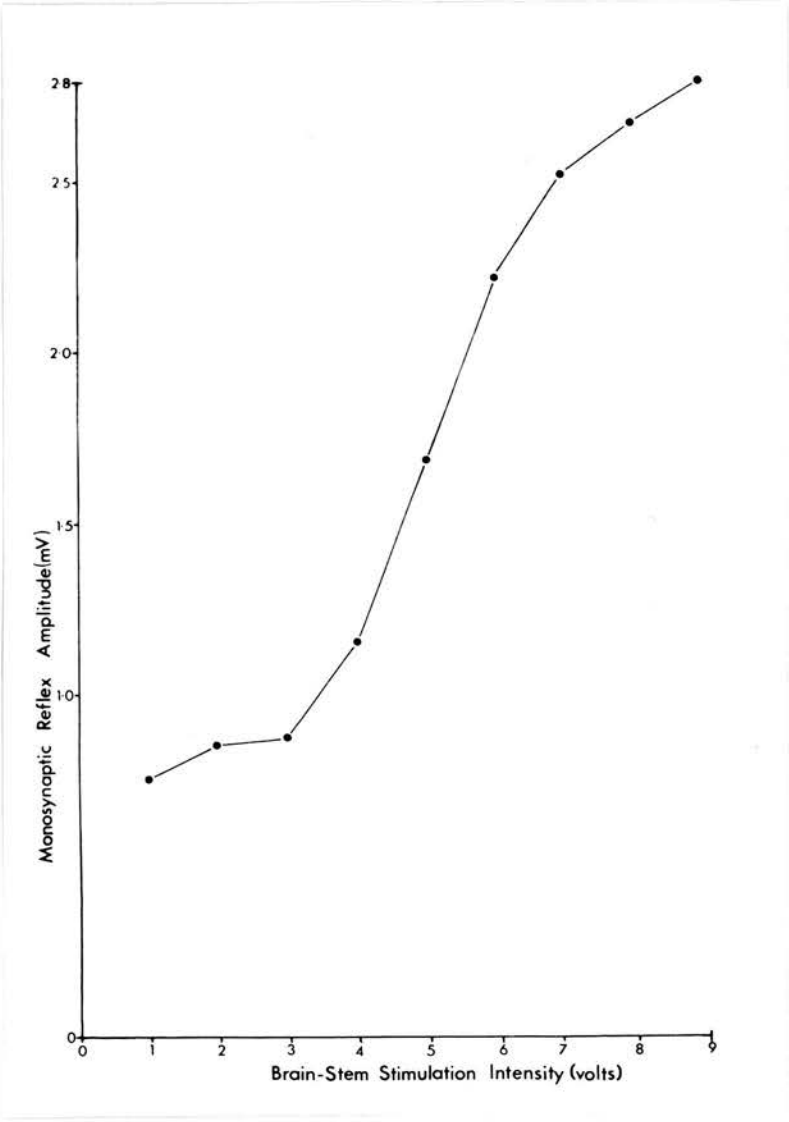

FIGURE 27

The effect of raphe-stimulating intensity on MSR amplitude. Each point represents the mean of three consecutively evoked MSRs.

Dorsal root stimulus intensity

In some animals the degree of MSR potentiation was related to the dorsal root voltage. In these instances, the higher dorsal root voltages were associated with greater potentiation. In the majority of animals however, apart from the lowest dorsal root voltages, the degree of MSR potentiation was independent of the dorsal root voltages. An example of this typical record is shown in Fig.28. In this figure the lower line of symbols (Δ) represents the amplitude of the unconditioned MSR at different dorsal root voltages. The upper line of symbols (o) represents the amplitude of the potentiated conditioned reflex. Each point represents the mean value of three consecutively evoked monosynaptic reflexes. At the higher dorsal root voltages, corditioning stimulation evoked a 150% increase in the MSR.

Monosynaptic reflex from unanaesthetised decerebrate preparations

The decerebrate rat has not proved to be an easy preparation to work with. On several occasions, the rat died within 30 minutes of making the mid-collicular lesion. Those animals that survived lived for between 4-6 hours. Although the decerebrate cat is characterised by a rather low blood pressure, the decerebrate rats generally became hypertensive. In most preparations some degree of extensor rigidity was evident, in others although the later histological examination of the brain stem confirmed the completeness of the lesion, extensor rigidity was never

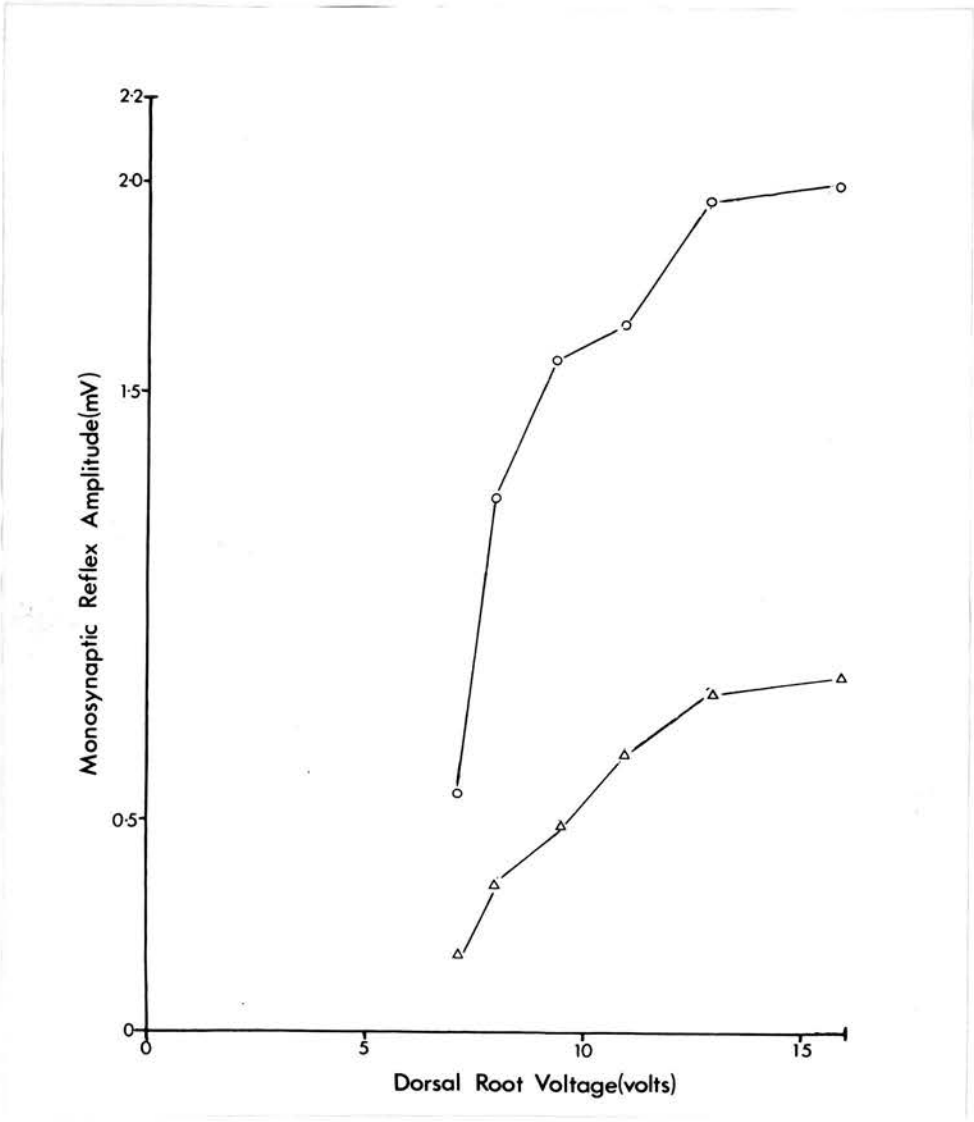

FIGURE 28

Effect of conditioning stimulation on the lumbar MSR. The lowermost symbol (△) represents the amplitude of the unconditioned MSR. The upper symbol (o) represents the conditioned MSR amplitude.

apparent. In the majority of the 20 decerebrate rats a muscle relaxant was administered (under supervision); this was essential in view of the spontaneous muscle movements associated with this type of preparation.

The monosynaptic reflex recorded from the lumbar spinal cord was very much greater in amplitude in the decerebrate rat than in the anaesthetised preparation. Although the normally observed maximum amplitude response associated with Fluothane (0.5%) anaesthetised rats was about 2mV, in the decerebrate preparation MSRs of up to 8mV were frequently recorded. However the configuration of the reflex potentials was directly comparable in the two preparations.

Histological examination of the brain stem of decerebrate preparations has confirmed that the lesion was anterior to the raphe nuclei.

The effect of conditioning stimulation was similar in both the decerebrate and the intact anaesthetised preparation. Thus although the amplitude of the unconditioned reflex was greater in the decerebrate preparation, conditioning stimulation at a supramaximal dorsal root voltage increased the MSR by about 70% (i.e. comparable to the potentiation recorded in anaesthetised rats).

Comparability of the MSR potentiation between animals

The extent of the potentiation of the MSR has been shown to be dependent upon a series of factors. However, even when allowances are made for recognized variables e.g. stimulation parameters, conditioning-dorsal root stimulus interval, exact location of stimulating electrodes and

dorsal root voltage, there still appears to be considerable between-animal variability in the MSR potentiation. These variations may well be explained in terms of the condition of the animal or motoneurone pool sample (see Discussion). For this reason, it does not seem justifiable to directly compare the percentage potentiation (i.e. the ratio of the potentiation to the maximum unconditioned MSR) from one animal to another.

However the more significant finding is that qualitatively similar effects of raphe stimulation on MSRs were recorded in almost all animals irrespective of the presence or absence of anaesthesia. Changes in the extent of the potentiation within one experiment associated with the administration of a drug may well be directly comparable and have been examined in the relevant section.

Polysynaptic reflex (PSR) activity

Polysynaptic reflex activity was recorded from ventral roots after the monosynaptic discharge had terminated. An example of PSR activity can be seen in Fig.23. The amplitude of the reflex was invariably smaller than the MSR, rarely exceeding 1 mV. The amplitude of the polysynaptic reflex was far less stable than the MSR. The reflex often deteriorated spontaneously during the course of the experiment at a time when the MSR amplitude was perfectly constant. This instability has limited the study of interneuronal activity. However from those studies successfully completed it is apparent that over a restricted range the reflex amplitude varies with the intensity of dorsal root stimulation.

In 11 rats in which the PSR activity was monitored, conditioning stimulation either profoundly reduced or abolished the reflex. This effect is illustrated by Fig.23, although a substantial PSR has been elicited by the unconditioned dorsal root stimulus, the conditioned reflex is virtually absent. Thus stimulation of the raphe nucleus which increases the MSR amplitude, reduces the PSR amplitude. The pharmacology of this effect has been investigated (see below).

Pharmacology of the raphe-spinal pathway

Stimulation of a region in the brain stem corresponding to the raphe nuclei has been shown to potentiate subsequently evoked MSRs. The aim of the experiment was to stimulate electrically the descending 5-HT pathway. However the problems associated with selectively stimulating fine fibre pathways indicated that a pharmacological identification of the descending effect was essential. Thus if 5-HT has been released as a result of the conditioning stimulation applied to the raphe nuclei, 5-HT antagonists would be expected to block the effect of conditioning stimulation. The following series of experiments describes the effect of 3 intravenously applied 5-HT antagonists on the MSR potentiation.

Effect of intravenous LSD

LSD was administered to 11 rats in doses ranging from 5ug-25µg/kg. The initial effect observed with the larger dose of LSD was a reduction in the amplitude of both the conditioned and the unconditioned MSR. However the dose

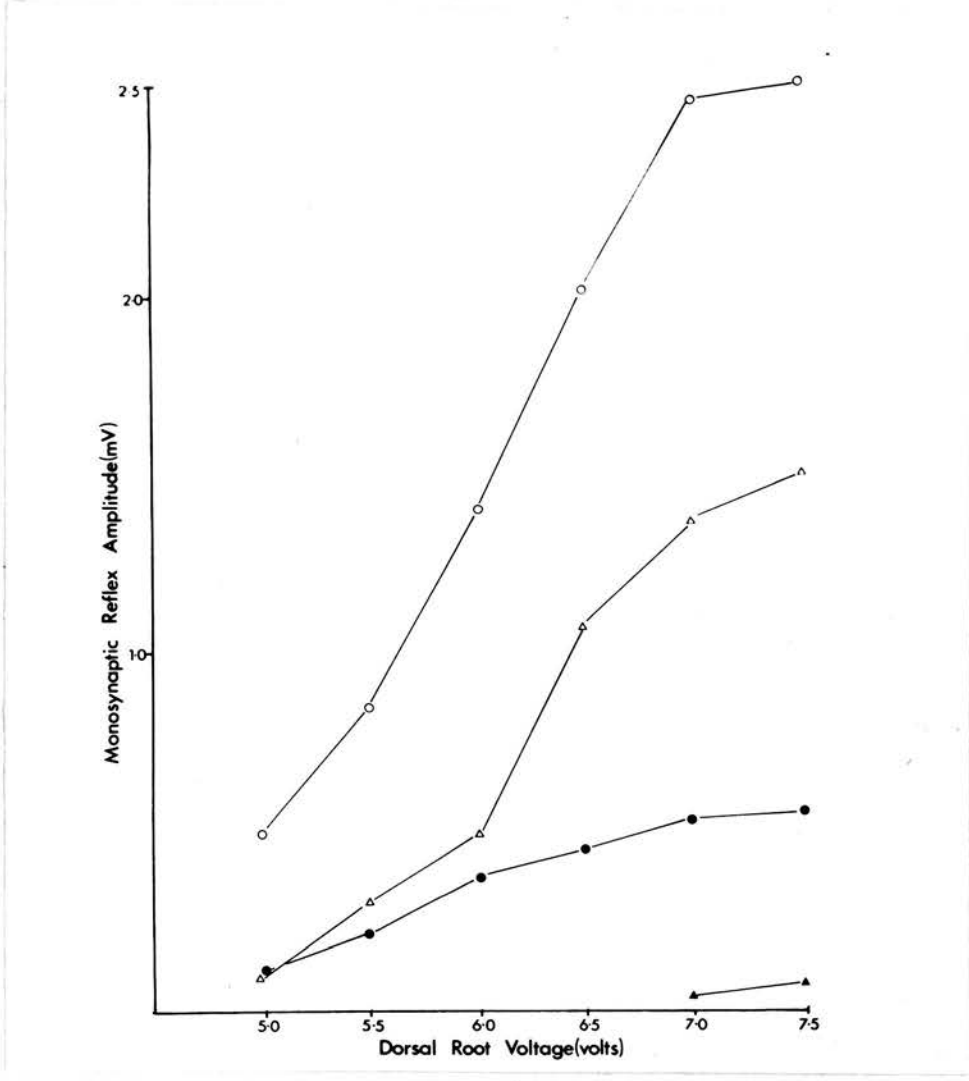

FIGURE 29

The effect of LSD (15µg/kg) on spinal reflexes. The open symbols (△) (o), represent the unconditioned and conditioned MSR. The closed symbols represent the reflexes after the administration of LSD. The antagonist has profoundly reduced the amplitude of all reflexes. Each point represents the mean amplitude of three consecutively evoked reflexes.

of LSD has been identified which although not reducing the unconditioned reflex amplitude, did profoundly reduce the potentiating effect of conditioning stimulation.

Fig.29 illustrates the general depressant effect of larger doses of LSD. The open circles and triangles represent the conditioned and unconditioned MSR amplitude respectively. The filled symbols represent the same reflexes after the administration of 15µg/kg LSD. Although there is some indication of a reduction of the effects of conditioning stimulation on the MSR, the predominant effect is a general reduction in excitability of the motoneurone pool. It is thus possible to explain the partial blocking of the MSR potentiation in this figure, in terms of a non-specific depressant action of LSD on neuronal activity.

By systematically reducing the administered dose of LSD, the dose which reduced the potentiating effect but not the unconditioned MSR has been identified. The slow intravenous administration of between 5-10µg/kg of LSD over a period of 2 minutes little affected the amplitude of the unconditioned MSR. Similarly the drug rarely produced any changes in blood pressure. However the drug reduced the potentiating effect of raphe stimulation by 75% in three animals. In other preparations, the potentiation was reduced by between 33-60%. The time course of the blocking effect is illustrated by Fig.30. Again in this preparation LSD (10µg/kg) has had little effect on the unconditioned MSR amplitude. Three minutes after the administration of the drug the MSR potentiation has been

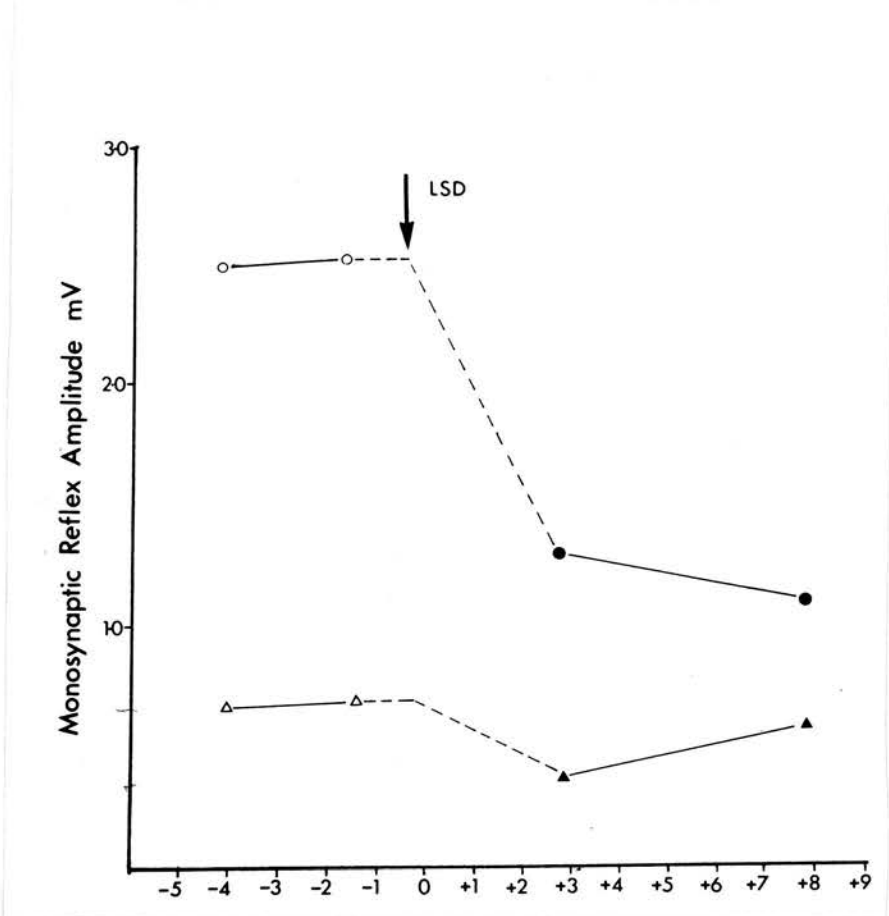

FIGURE 30

The effect of intravenous LSD (10 µg/kg) on the unconditioned (Δ) and conditioned (o) MSR. Three minutes after the intravenous adminstration of the antagonist, the conditioned MSR is 50% smaller. The unconditioned MSR has been less reduced in amplitude. Each point represents the mean amplitude of three consecutively evoked potentials.

reduced by 50%, 5 minutes later the potentiation had been reduced by 66% and at this point the amplitude of the unconditioned MSR is almost exactly as great as the pre-drug level.

This graph is constructed from the results of an experiment in which the effect of LSD was being examined on MSRs of different amplitude. As with all similar graphs presented here, each point represents the mean amplitude of three consecutive reflexes evoked at 4 second intervals. Although it would have been more satisfactory to test the MSR amplitude immediately before the administration of the antagonist, this was not possible in view of the experimental design.

The time course of the recovery of the potentiation after blockade was followed in 3 rats. After a dose of between 8-10µg/kg LSD the initial MSR potentiation was observed 160-200 minutes after the introduction of the drug. In two preparations the recovery of the potentiation was followed by a rebound increase in the degree of MSR potentiation.

In two other animals the MSR potentiation was not only reduced in amplitude but the effect of raphe stimulation was reversed. Within 5 minutes of administering 5.0µg/kg LSD to these animals, potentiation of the MSR had been reduced. Between 30 and 50 minutes later the conditioning stimulation applied to the raphe nuclei inhibited the MSR. During this period no evidence of MSR potentiation was recorded. Between 100 and 120 minutes after the antagonist had been administered

the original potentiation of the MSR was recovered.

Effect of intravenous Cinanserin

Cinanserin, regarded as having both peripheral (Rubin et al. 1964) and central (Roberts and Straughan 1967) 5-HT antagonist properties, has been intravenously injected into 6 rats. At a dose of between 3-4 mg/kg, cinanserin reduced the potentiation of the MSR between 25-80%. The effect was observed within 10 minutes of applying the drug and was maximal after 15-30 minutes. In two experiments full recovery of the MSR potentiation was achieved between 60-90 minutes after the drug administration. As with LSD higher doses (above 4mg/kg) of the antagonist reduced the unconditioned MSR amplitude, however the sensitivity of the reflex pathway to cinanserin was considerably less than to LSD.

Effects of intravenous Methysergide

Methysergide (0.75-1.0 mg/kg) was administered to 4 rats. At this dose the antagonist produced only a very small fall in the amplitude of the unconditioned MSR. The antagonist was however effective in blocking the brain stem evoked potentiation of the MSR. In 4 preparations the average reduction of the potentiation was 59%, ranging from 30% to 70%. Injection of the drug was not accompanied by any change in blood pressure. Onset of drug effects on reflex activity was quite rapid. Within 10 minutes, the blocking action was well developed (see Fig.31). Recovery of the potentiation was evident to some extent in all experiments and complete in two preparations about 45 minutes after the drug had

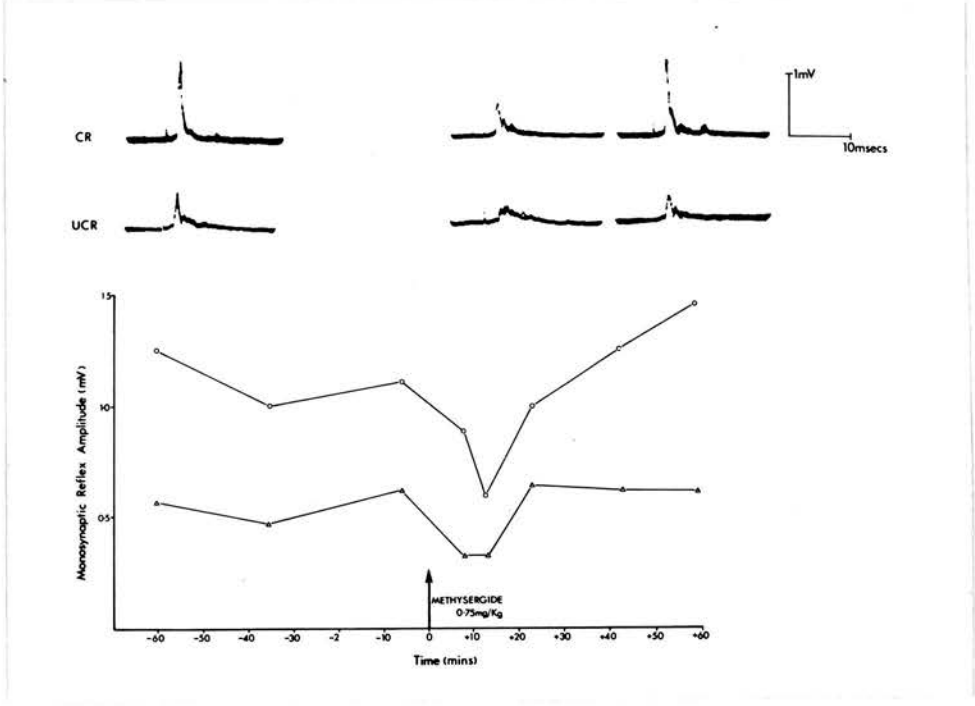

FIGURE 31

The effect of intravenously administered methysergide (.75 mg/kg) on the unconditioned (Δ) and conditioned (o) MSR. About 10 minutes after injecting the antagonist, the blocking action was well developed. Note the minimal effect of methysergide on the unconditioned MSR.

been administered. From Fig.31 it is evident that there is a slight rebound increase in the potentiating effects of conditioning stimulation following recovery from the drug effects. In the upper half of the figure are illustrated the original reflex potentials from which the graph is constructed.

Effect of L-tryptophan on reflex activity

Since L-tryptophan is a precursor of 5-HT, its administration will increase 5-HT levels in the CNS. If the MSR potentiation elicited by raphe stimulation involves neurotransmission at a 5-HT synapse, then the administration of L-tryptophan might be expected to modify the potentiation.

Being extremely insoluble, the precursor was emulsified with Tween 80, dissolved in injection saline and subsequently administered either intravenously or intraperitoneally to 14 rats. Doses of L-tryptophan ranged from 80-800 mg/kg.

In the majority of animals (12 rats) L-tryptophan produced a gradual increase in the amplitude of the unconditioned MSR over a period of 1-3 hours. This increase, in the range 20-80% above the pre-tryptophan amplitude can be seen in Fig.32. In this figure the circles represent the amplitude of the unconditioned MSR, the triangles represent the conditioned reflex amplitude.

No consistent effect of the precursor on the conditioned MSR amplitude was noted until about 90 minutes after the drug had been applied. As can be seen from Fig.32, after this time interval the amplitude of the conditioned MSR increased dramatically. Within 90-150 minutes the extent of

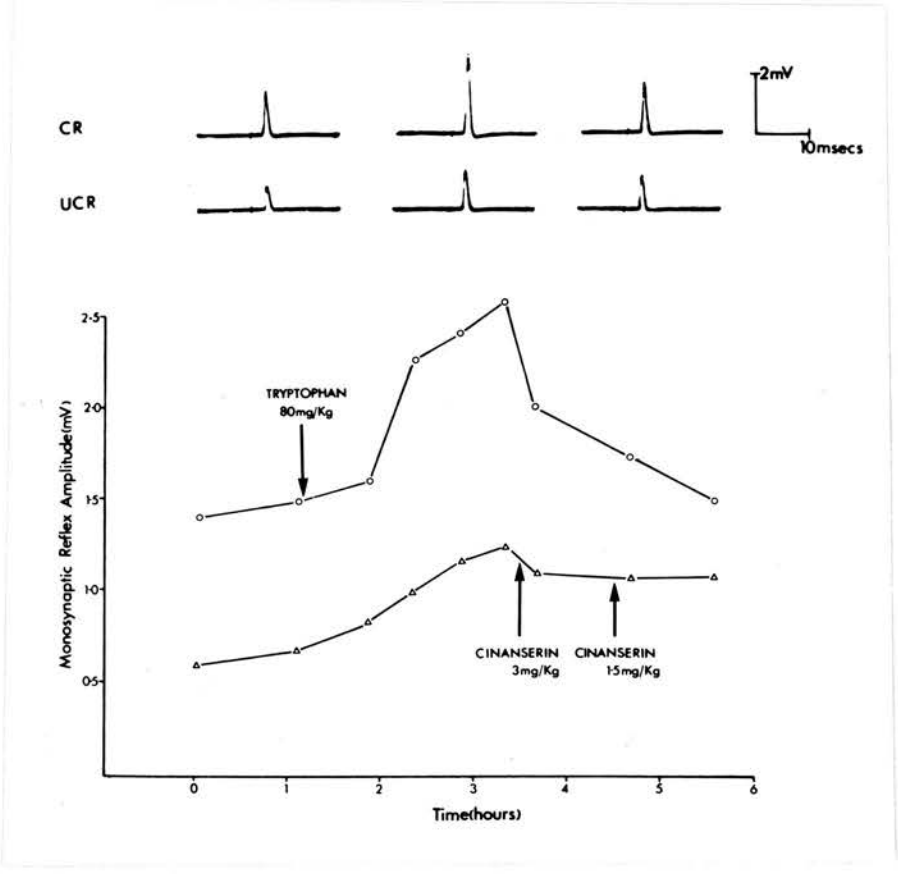

FIGURE 32

Effect of L-tryptophan (80 mg/kg i.v.) and cinanserin on the unconditioned MSR amplitude (Δ) and the conditioned MSR amplitude (o). About 90 minutes after injecting the precursor, the conditioned MSR increased in amplitude. The i.v. injection of the 5-HT antagonist cinanserin, reversed this trend. Some of the original reflex potentials from which the graph was constructed are illustrated above. The potentials evoked in the absence of raphe stimulation (the unconditioned reflex, UCR) are arranged along the bottom line, the conditioned reflexes (CR) along the top line.

the MSR potentiation has increased by 100% above pre-drug values. Thus although L-tryptophan has caused an increase in the amplitude of the unconditioned MSR, the increase in the conditioned reflex amplitude is much greater. A dorsal root stimulus intensity corresponding to 50% maximum MSR has been chosen in Fig.32 Examples of the changes in the reflex amplitude which occurred during the experiment are illustrated in the upper half of the figure. The reflexes are placed approximately above that part of the graph to which they correspond. The gross changes in reflex amplitude are apparent from the traces. No polysynaptic reflex was recorded in this particular experiment.

The ability of L-tryptophan to increase the MSR potentiating efficacy of raphe stimulation has been observed in all but two preparations. In these two preparations the precursor did not affect the reflex activity. Small variations in the time course of the effect have been noted corresponding to the different routes of administration. As might be expected the intravenous route proved to be more effective in terms of latency to onset of effects. Control experiments have been performed to examine any possible effects of either the emulsifying agent or injection saline on reflex activity. The intravenous injection of both substances produced no modification of spinal reflex activity.

L-tryptophan has been administered intravenously (80-200 mg/kg) and intraperitoneally (200-800 mg/kg). Although the lower intravenous dose was sufficient to cause a supramaximal change in reflex activity, a dose of between

300-400 mg/kg was required when the drug was administered intraperitoneally.

The continual recording of blood pressure throughout the experiment has allowed any effects of administered drugs on the cardiovascular system to be assessed. The intraperitoneal route of administration never caused any fluctuations in blood pressure. The rapid intravenous injection of L-tryptophan was observed to increase blood pressure. However by injecting the precursor over a period of 5 minutes, blood pressure changes were not greater than 15-20 mm.Hg and rapidly recovered to resting levels.

Fig.33 illustrates the effect of L-tryptophan on both the unconditioned (lower symbol) and conditioned (upper symbol) reflex evoked at different dorsal root voltages. The open circles represent the averaged responses recorded before the precursor was administered. The maximum reflex amplitudes (both conditioned and unconditioned) appear to have been reached at a dorsal root voltage corresponding to 11 volts (about 0.23 mA).

The triangles represent the reflex responses 60 minutes after the intraperitoneal injection of L-tryptophan (300 mg/kg). Several interesting features are now apparent. Firstly, at the lower dorsal root voltages, both the unconditioned and the conditioned MSRs are profoundly depressed. However even at this lower level of motoneurone pool responsiveness, the degree of potentiation suggests an increased efficacy of conditioning stimulation.

At the higher dorsal root voltages, L-tryptophan has

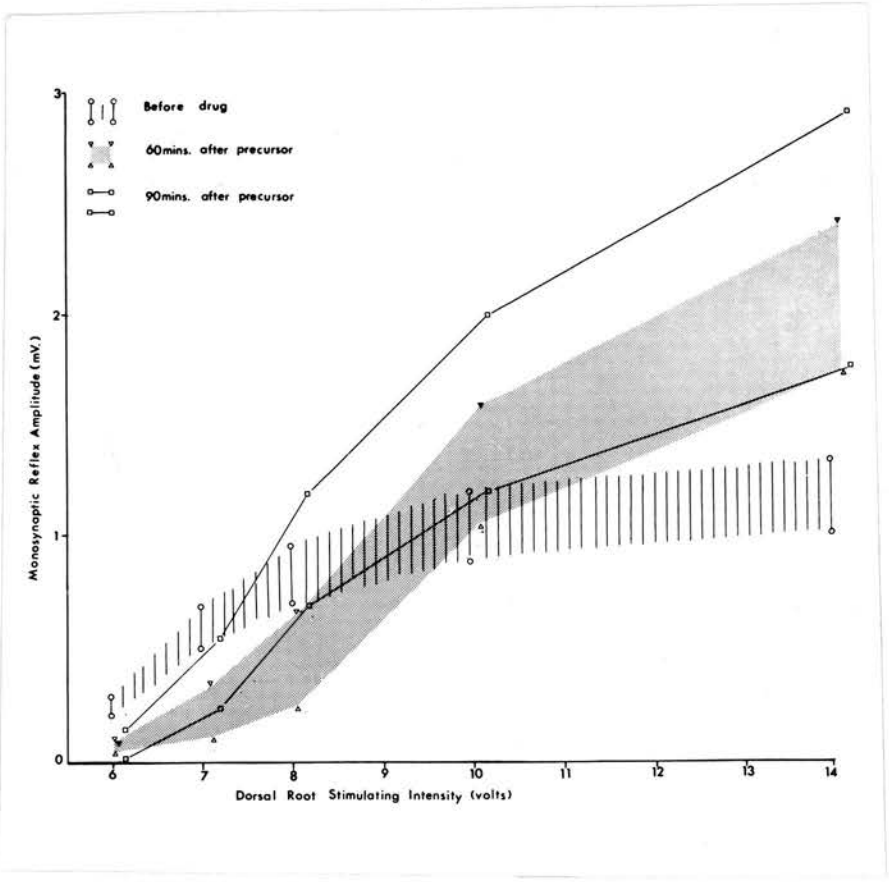

FIGURE 33

The effect of L-tryptophan (300 mg/kg i.p.) on the MSR amplitude evoked at different dorsal root stimulating intensities. At the lower dorsal root stimulating intensities the precursor reduces the amplitude of both the unconditioned and conditioned MSR. Conversely at the upper end of the stimulus response curve, L-tryptophan increases both the unconditioned and conditioned MSR.

Each point represents the mean of three consecutively evoked reflexes.

not reduced the unconditioned MSR amplitudes and the degree of potentiation after 60 minutes has already increased by about 100%.

The squares represent the reflex amplitudes 90 minutes after the drug had been administered. At the two lower dorsal root voltages the reflexes are still reduced in amplitude, but not to the same extent as the 60 minute values.

The MSR amplitude corresponding to a dorsal root voltage of 9 volts is especially interesting. After 90 minutes the unconditioned MSR amplitude is exactly similar to the pre-drug reading, yet the conditioned reflex is considerably greater in amplitude. Thus although the motoneurone pool is at a similar level of excitability, the efficacy of the conditioning stimulation is considerably greater 90 minutes after the administration of the precursor.

The increase in efficacy of conditioning stimulation is even more apparent at supramaximal dorsal root voltages, although at such stimulus intensities the unconditioned MSR amplitude is well above pre-drug levels.

L-tryptophan was not observed to affect the latency of the MSR. In 3 preparations spontaneous activity was recorded in the ventral root between 60 and 90 minutes after the administration of the precursor.

An increase of up to 2°C in the rectal temperature was observed in 4 preparations. The changes in reflex amplitude reported above are however not dependent upon an increased body temperature as in the majority of experiments the rectal temperature was held constant.

Action of 5-HT antagonists on the monosynaptic reflex in L-tryptophan pretreated rats.

In 4 rats pretreated with L-tryptophan, 5-HT antagonists were administered at a time when the effect of the precursor on the MSR potentiation was nearly fully developed. Fig.32 illustrates the blocking action of cinanserin (3 mg/kg) on the MSR potentiation. Within 45 minutes of giving the antagonist, the potentiation had been reduced to 50% of the tryptophan-induced increase, although the blocking action of the antagonist commenced usually within 10-20 minutes depending upon the dose.

A second smaller dose of antagonist reduced the MSR potentiation to a level which was 50% less than the pre-tryptophan level. Thus the antagonist had completely reversed the facilitating effect of the precursor and had blocked the potentiating effects of raphe stimulation per se.

The reflex potentials illustrated above the graph correspond to changes in amplitude of the MSR before and after administration of the precursor and also after injection of the antagonist. Each potential represents three superimposed consecutively evoked reflexes. The unconditioned reflexes (UCR) are presented along the bottom line, the conditioned reflexes (CR) along the upper row.

Effect of 5-HT antagonists on polysynaptic reflex

The problems associated with the long-term monitoring of PSR have already been mentioned. However in 2 experiments in which 5-HT antagonist was administered, the unconditioned PSR amplitude remained constant up to the time of drug

administration. On one occasion LSD (10µg/kg) was administered, on the other cinanserin (4 mg/kg).

Six minutes after the LSD injection, the inhibitory influence of conditioning stimulation on the PSR had been reduced by 50%; after a further three minutes, conditioning stimulation produced a slight potentiation of the PSR. The normally observed inhibitory effect was recovered 15 minutes after the drug had been applied. The recovery of the original inhibitory influence of conditioning stimulation suggests that the blocking effect attributed to LSD is a real one and not due to the steady deterioration of the preparation.

Effect of drugs on spinal reflexes in the decerebrate preparation

Conditioning stimulation applied to the raphe nuclei in decerebrate rats had qualitatively similar effects on the MSRs recorded from ventral roots (see above). The average effect of LSD in the decerebrate preparation was to reduce potentiation by 65%. This falls well within the range recorded with Fluothane-anaesthetised rats. Similarly the effects of the other antagonists, methysergide and cinanserin, were also qualitatively similar to those recorded in anaesthetised preparations.

These observations confirm that the potentiating effect of conditioning is not mediated via higher centres in the brain (see Discussion).

Field Potential Recording

A second method of recording spinal reflex activity involves the monosynaptically evoked responses of either one

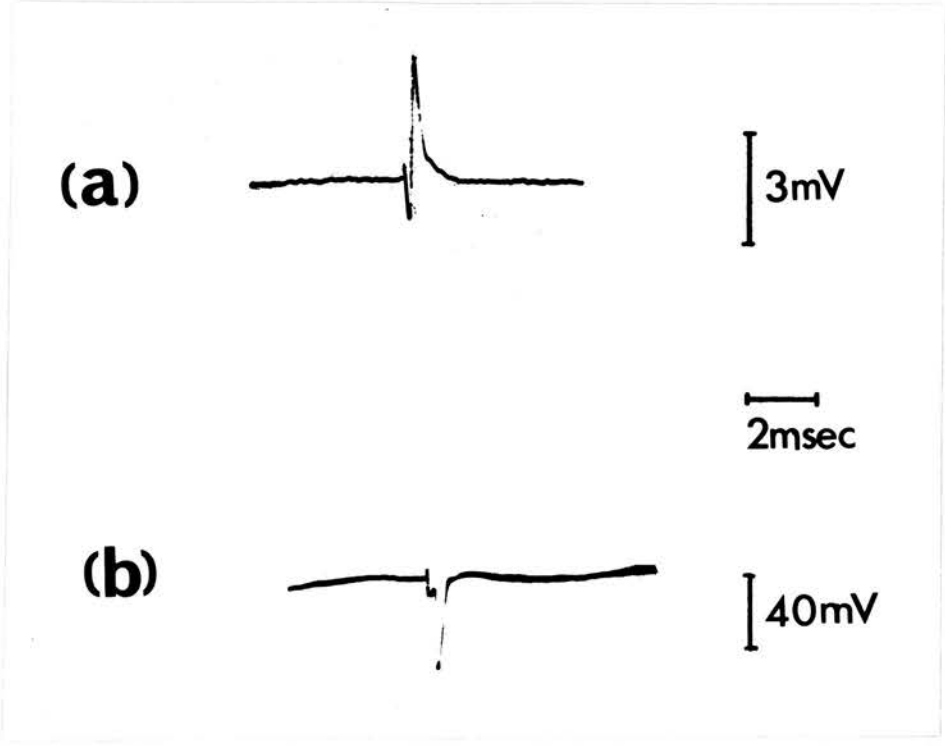

FIGURE 34

The upper trace a) is an extracellular field potential recorded close to a motoneurone in the lumbar spinal cord of rat. The field potential was elicited by the antidromic stimulation of the cut ventral root. The latency to onset of the field potential is about 0.75 msecs. After moving the microelectrode tip 50μ more ventral the lower trace b) was recorded. This is an intracellular recording from a lumbar motoneurone. The action potential was evoked by antidromic stimulation of the cut ventral roots.

or a small group of motoneurones. This can be achieved using recording microelectrodes located within the ventral horn of the spinal cord. Initially the position of a motoneurone has been identified by the antidromic stimulation of the ventral roots. The resulting action potential invades motoneurones and it is this activity which is recorded as an antidromic extracellular field potential.

Fig.34 illustrates an extracellularly recorded potential evoked by stimulation of the ventral root.(upper trace). Both the amplitude and the configuration of the field potential were observed to vary according to the relative position of the microelectrode with respect to the motoneurone pool. As the microelectrode progressed down through the spinal cord, the amplitude of the antidromically evoked field potential initially increased, reached a maximum, and subsequently became smaller.

Fig 35 illustrates these changes in the recorded field potentials. Each trace consists of 3 superimposed evoked potentials. The accompanying graph represents the relative amplitudes of the unconditioned and conditioned (see later) field potential at different depths (abscissa) within the spinal cord. From the graph it is evident that the largest field potential in this particular track was recorded at a depth of 1850-1950μ. It can thus be concluded that the recording microelectrode either passed very close to a motoneurone at that depth or that several motoneurones were located at that depth.

The optimum recording position was regarded as one

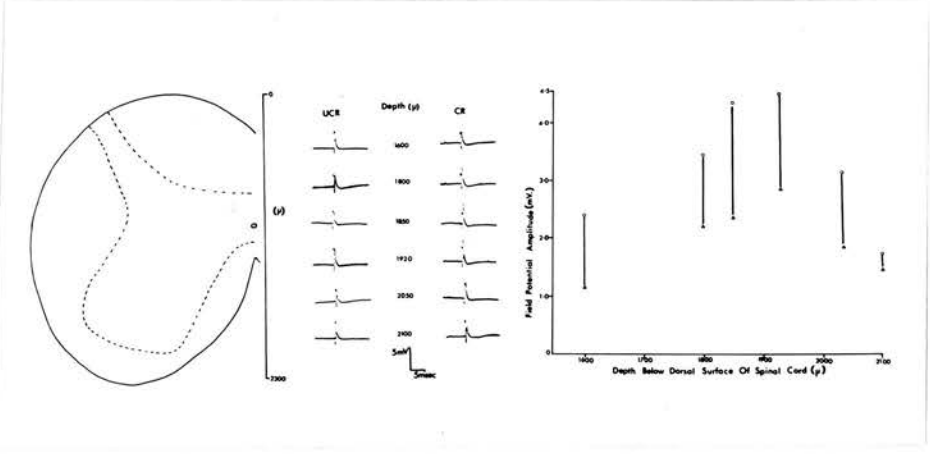

FIGURE 35

Extracellular field potential recorded at different depths from the ventral horn of the lumbar spinal cord of rat. The field potentials were evoked by stimulation of lumbar ventral roots. Conditioning stimulation applied to raphe nuclei increased the amplitude of the field potential (see middle section of figure). The extent of the potentiation at different depths in the ventral horn is illustrated by the graph. The potentiating effect is most pronounced at the depth corresponding to the maximal unconditioned (△) field potential amplitude.

from which the largest possible antidromic field potential (usually 3-4 mV) was recorded. Fig.34 illustrates an extracellular field potential (upper trace) recorded from the ventral horn of a rat spinal cord. After moving the microelectrode 50μ further into the ventral horn, the electrode entered a motoneurone and the action potential shown in the lower trace was recorded. The latency to onset of the extracellular field potential is 0.75 msec. This value was obtained by extrapolating the trace base line to the point at which it met the upward sweep of the response. The existence of an early positive component suggests the field potential may have commenced slightly earlier.

The maximum amplitude of the extracellular field potential recorded just outside the cell, occurs only about 0.08-0.10 msec. after the intracellularly recorded peak. This suggests that the cell from which the intracellular recording was made, was being activated antidromically slightly earlier than the majority of motoneurones contributing to the field potential.

Locating the motoneurone pool corresponding to the stimulated ventral root was not difficult. Although ventral roots generally run under the cord some distance before entering the CNS, with experience it was possible to accurately predict the position of the motoneurone pool. Having identified the position within the ventral horn from which the maximum antidromically evoked field potential was recorded, the corresponding dorsal root was stimulated, eliciting a monosynaptic reflex.

The orthodromically evoked monosynaptic reflex has proved rather more difficult to identify. The negative field potential associated with the reflex discharge was usually superimposed on a complex positive waveform. The average transients computer has been used to identify the constancy of the orthodromic potential. Fig.36 (B) (upper trace) illustrates the recording of 10 averaged orthodromically evoked field potentials. The early negative potantial may be due to activity in neighbouring afferent fibres. The potential due to orthodromic stimulation is seen as an inflection on a positive potential. Confirmation that this potential was indeed a manifestation of the orthodromically evoked MSR is provided by Fig.36 (A). The middle trace in this figure illustrates the normally observed field potential. The lower trace is an intracellular recording obtained from the same region as the field potential. The peak amplitude of the orthodromically evoked intracellular spike coincides temporally with the field potential. It is clear therefore that the recorded field potential was the extracellular manifestation of motoneurone activity. The upper trace of Fig.36 (A) illustrates the MSR recorded from ventral roots and evoked by stimulation of the dorsal roots. The time course of the three potentials is similar. Measurements of the variations in the amplitude of the orthodromic field potential have been made with respect to the trace baseline. Thus the amplitude of the potential was regarded as the distance between the peak of the field potential and the oscilloscope trace baseline.

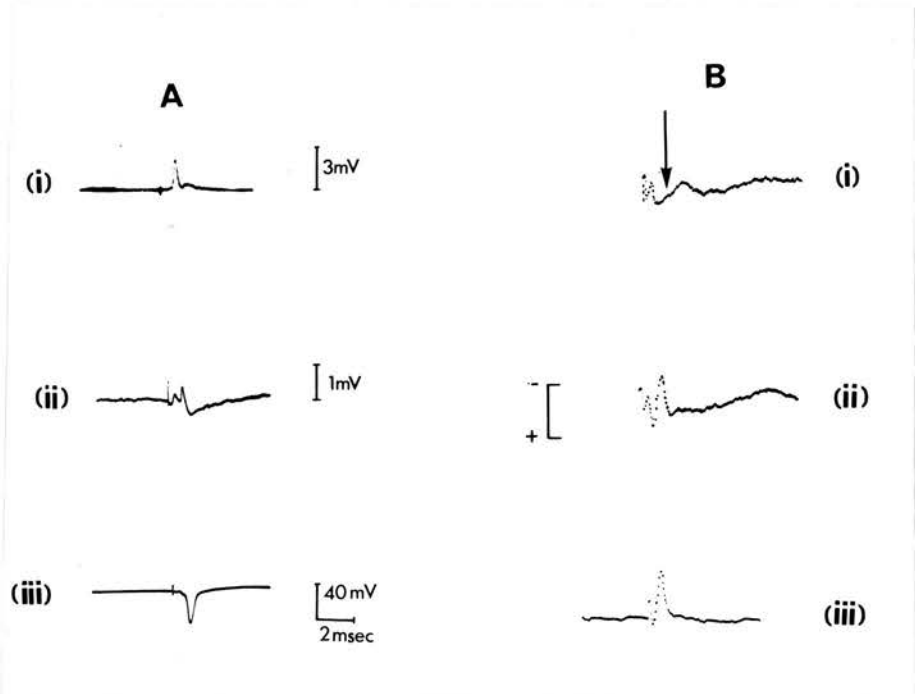

FIGURE 36

A (i-iii), comparison of a monosynaptic reflex evoked by stimulation of lumbar dorsal root and recorded from (i) a ventral root, (ii) recorded extracellularly from the lumbar motoneurone pool and (iii) recorded intracellularly from a lumbar motoneurone.

The effect of conditionig stimulation on the orthodromic extracellular field potential is illustrated by B (i-iii), each trace represents the averaged response of 10 evoked reflexes. Trace B (i) represents the reflex in the absence of conditioning stimulation. The arrow marks the position of the orthodromic potential. The middle trace was evoked immediately after conditioning stimulation. The orthodromic field potential is much greater in amplitude. The lower trace was obtained by subtracting the computed unconditioned response from the conditioned response. The resulting trace represents purely the potentiating effect of conditioning stimulation on the orthodromic field potential. The absence of any other component of the complex field potential in trace B (iii) confirms that only the portion of the complex marked by the arrow in trace B (i) may be modified by conditioning stimulation.

Effect of raphe nuclei (conditioning) stimulation on field potential recordings

The application of conditioning stimulation to the raphe nuclei (using standard stimulation parameters) increased the amplitude of both the antidromically and orthodromically evoked potentials. These observations have been made in a series of 20 separate studies obtained from 12 rats.

Although the amplitude of the orthodromic potential was initially much smaller than the antidromic potential, conditioning stimulation frequently increased the former potential by up to 400%, whereas the antidromic potential was only increased by about 50%.

Changes in the antidromic field potential after conditioning stimulation are illustrated in Fig.35. From the graph it can be seen that the extent of the potentiation depends partly upon the relative position of the microelectrode and the motoneurone pool. Thus the larger the field potential (and hence the nearer the microelectrode is to either a single or a group of motoneurones), the greater is the degree of potentiation. In most studies the intensity of conditioning stimulation has been constant at between 2-3 volts (corresponding to a current of 40-100μA). This current represents a stimulus intensity well below that required for maximal potentiating effects.

The potentiating effect of conditioning stimulation on the orthodromic potential is illustrated in Fig 36 (B). The centre trace in this figure represents the computed average of 10 evoked potentials. The extent of the

potentiation is apparent when this trace is compared with the upper trace. The lower trace represents purely the effects of conditioning stimulation on the orthodromic field potential. The trace was obtained by subtracting the computed unconditioned response from the conditioned response. The resulting trace confirms the constant nature of the standing wave form and indicates clearly the potentiating effect of conditioning stimulation on the orthodromic field potential. If the potentiating effects of conditioning stimulation on the field potential are due to activity of 5-HT close to motoneurones, it may be predicted that such effects will be modified by drugs which interfere with the normal pharmacology of 5-HT. The following experiments describe the effect of iontophoretically applied drugs on the unconditioned and conditioned field potentials.

5-Hydroxytryptamine

5-HT has been iontophoretically applied during 6 studies involving 6 different recording situations. Each time 5-HT has been applied, the amplitude of the orthodromic conditioned field potential has been increased. In comparison the effect on the unconditioned response was very much less profound and less consistent. The unconditioned response increased in amplitude on 4 occasions, decreased twice and was unaffected on 6 occasions. Graphs relating changes in field potential amplitude during the application of drugs have been constructed by plotting the difference in amplitude between the conditioned and unconditioned field potential. In this way the graphs illustrate the modification of conditioning effects by the applied drugs.

An increase in the amplitude of the conditioned orthodromic field potential was recorded between 30 seconds and 1 minute after the 5-HT ejecting current (100 nA) had been applied. Fig.37 represents the changes in the difference in amplitude between the conditioned and unconditioned orthodromic potential during the iontophoretic application of 5-HT. The abscissa is divided into 2 minute intervals, the ordinate represents the difference in amplitude between the conditioned and unconditioned reflex amplitude in mVs. The drug has, in this instance, been applied for a period of 6 minutes.

Although there is a slight increase in the effect of conditioning stimulation within 1 minute of the drug application, a more profound effect is observed 3-5 minutes after the drug application had commenced. This initial slow onset of response followed by a rapid increase in response amplitude, typified the response of the conditioned field potential to 5-HT. Since the difference in field potential amplitude is still increasing at the end of the 5-HT application, it is probable that the maximum effect has not developed at this point.

During three applications of 5-HT the increased response amplitude was maintained for up to one minute after the drug application had been discontinued. However in the majority of drug studies the drug response began decaying as soon as the drug application was discontinued. From Fig.37 it can be seen that the response decays very rapidly 1-2 minutes after the termination of the drug application.

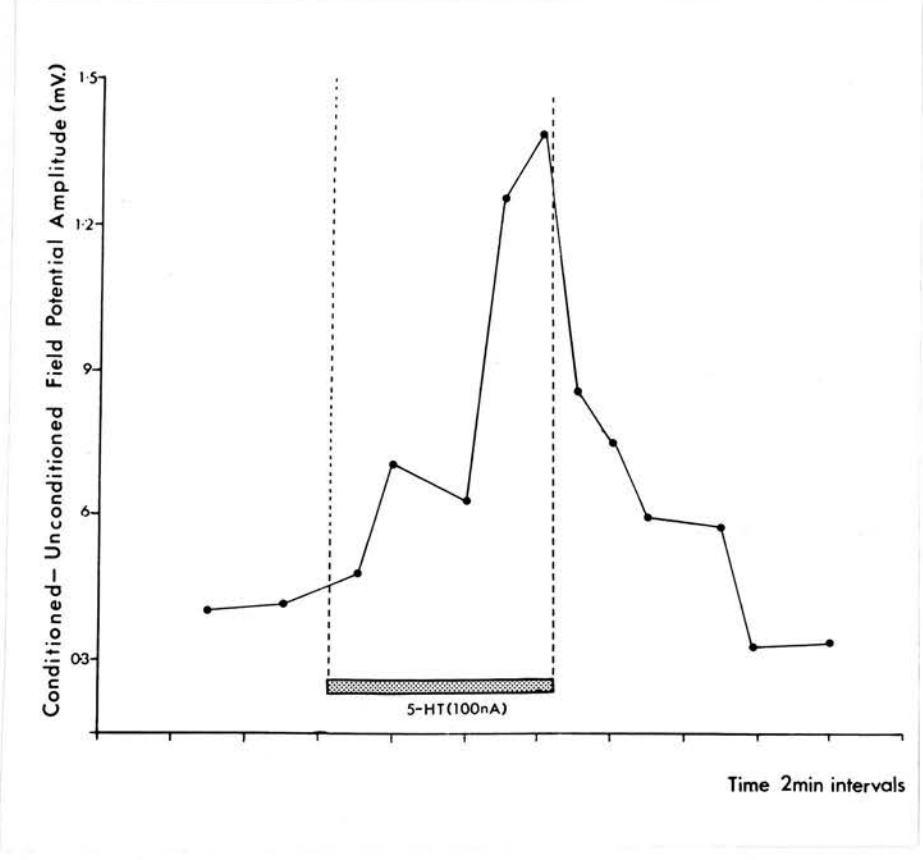

FIGURE 37

The effect of iontophoretically applied 5-HT (100 nA) on the difference in amplitude between the conditioned and the unconditioned orthodromically evoked field potential. The period of application of 5-HT is indicated by the bar along the abscissa.
Each point represents the mean of five consecutively evoked field potentials.

The field potential usually attains pre-drug levels about 3 minutes after the initial decay. Although in this drug study the pre-drug field potential amplitude was attained 6 minutes after the termination of drug application, in some studies a new post-drug field potential amplitude was achieved (e.g. see Fig.39).

All iontophoretic applications of 5-HT were accompanied by the application of a current of similar intensity but opposite polarity, thus reducing the possibility of current effects (see Materials and Methods). The slow onset of the recorded effects strongly denies the possibility that changes in current were responsible for the observed effects. No attempt has been made to construct dose-response curves, although it has been noted that a larger ejecting current of 5-HT produces bigger responses than a smaller current.

Cinanserin

The 5-HT antagonist cinanserin has been iontophoretically applied in six different recording situations involving 5 rats. As with 5-HT itself, cinanserin had a very positive effect on the conditioned orthodromic field potential. Each time the drug was applied the field potential was reduced in amplitude. The effect of the antagonist on the difference in amplitude between the conditioned and unconditioned field potential is illustrated in Fig.38. The time course of the changes in amplitude of the field potential is similar to that observed for the 5-HT response. Thus the maximum reduction in amplitude was observed about 2 minutes after the drug application had ceased.

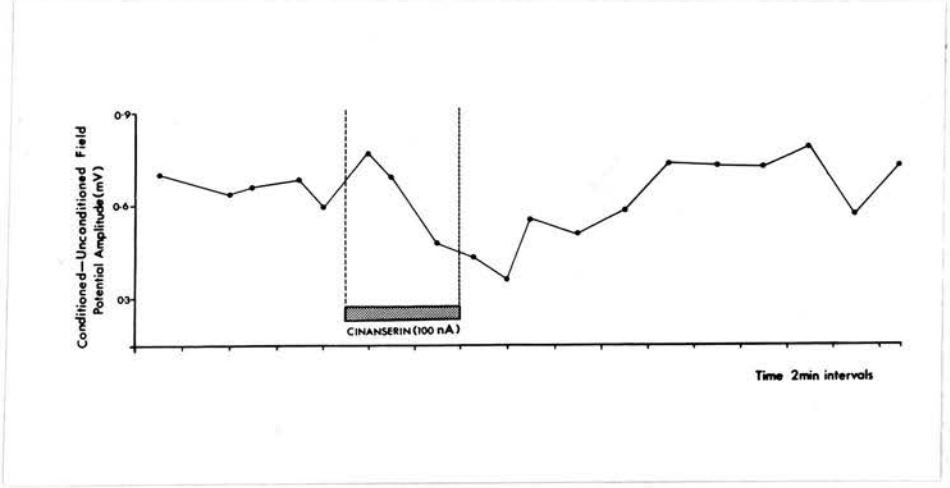

FIGURE 38

The effect of iontophoretically applied cinanserin (100nA) on the difference between the conditioned and unconditioned orthodromically evoked field potential. The period of application is indicated by the bar along the abscissa.

Recovery of the pre-drug amplitude was frequently extended over a 10 minute period The unconditioned orthodromic field potential was little affected by the antagonist, small increases and decreases in the amplitude being recorded at different times.

The blocking of the effects of conditioning stimulation on the field potential illustrated in Fig.38 is important. The observation that a 5-HT antagonist has prevented the potentiating effects of conditioning stimulation strongly suggests that such effects may be mediated via 5-HT close to motoneurones.

In a study extending over a period of 80 minutes, 5-HT was applied before, during and after application of cinanserin. The initial iontophoretic application of 5-HT produced the normally recorded increase in difference between the unconditioned and conditioned field potential amplitudes (Fig.39). Five minutes after the termination of the 5-HT application (and before the response had re-attained pre-drug levels) cinanserin application was commenced. The antagonist was applied for 25 minutes. During this application 5-HT was applied for two 5-minute periods. The second application (6 minutes after the beginning of the cinanserin application) produced a response comparable in configuration to the initial response. This response did however decay more rapidly than the first response to 5-HT. Twenty minutes after the beginning of the cinanserin application, a third application of 5-HT was made. The field potential amplitude remained unaffected

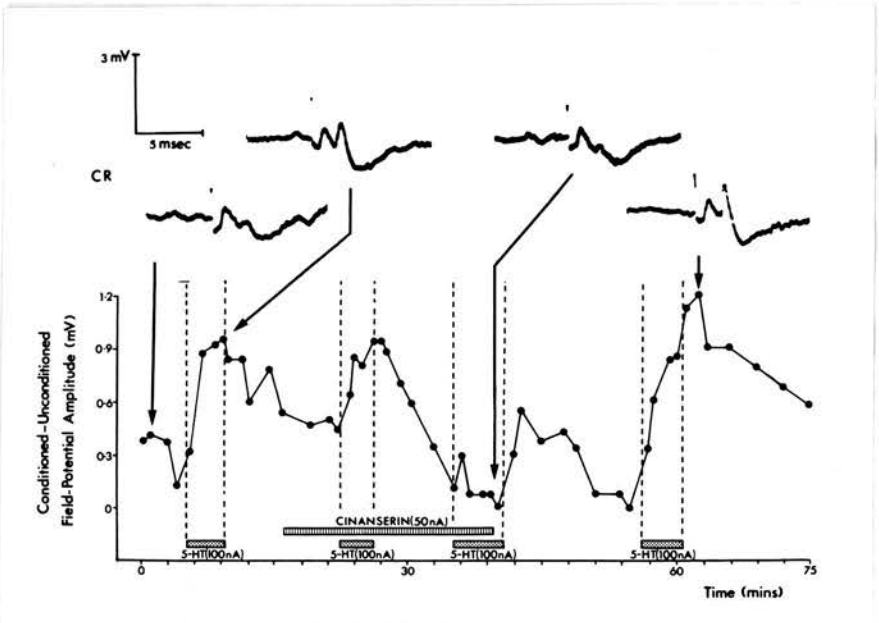

FIGURE 39

The effect of iontophoretically applied 5-HT (100 nA) and cinanserin (50 nA) on the difference between the conditioned and unconditioned orthodromically evoked field potential. 5-HT increases the effect of conditioning stimulation on the field potential. This effect is substantially reduced by the application of cinanserin.

during the application, however about 2 minutes after both 5-HT and cinanserin ejecting currents had been switched off, the potential showed an increase in amplitude. In addition, this third 5-HT response differed from previous responses in that the amplitude was much reduced.

An indication of the recovery of the system from the effects of cinanserin is provided by the fourth and last application of 5-HT. This application, 15 minutes after the termination of the current ejecting cinanserin, evoked a very large increase in field potential amplitude, the maximum response was attained 2 minutes after the termination of drug application. The traces shown above the graph in Fig.39 are recordings made during the experiment. The traces illustrate the modification of the conditioned orthodromic field potentials by 5-HT and cinanserin.

Effect of drugs on the antidromic field potential

Although the majority of drug studies have involved the modification of orthodromically evoked field potentials, both 5-HT and its antagonists have been iontophoretically applied on several occasions to the antidromic field potential.

5-Hydroxytryptamine

In 4 studies, 100 nA. of 5-HT has been applied over a 5-minutes period. Both the change in amplitude of the field potential and the time course of the response are comparable with the effect of the drug on the orthodromic field potential. In both cases the conditioned field potential was increased by about 0.6 mV.

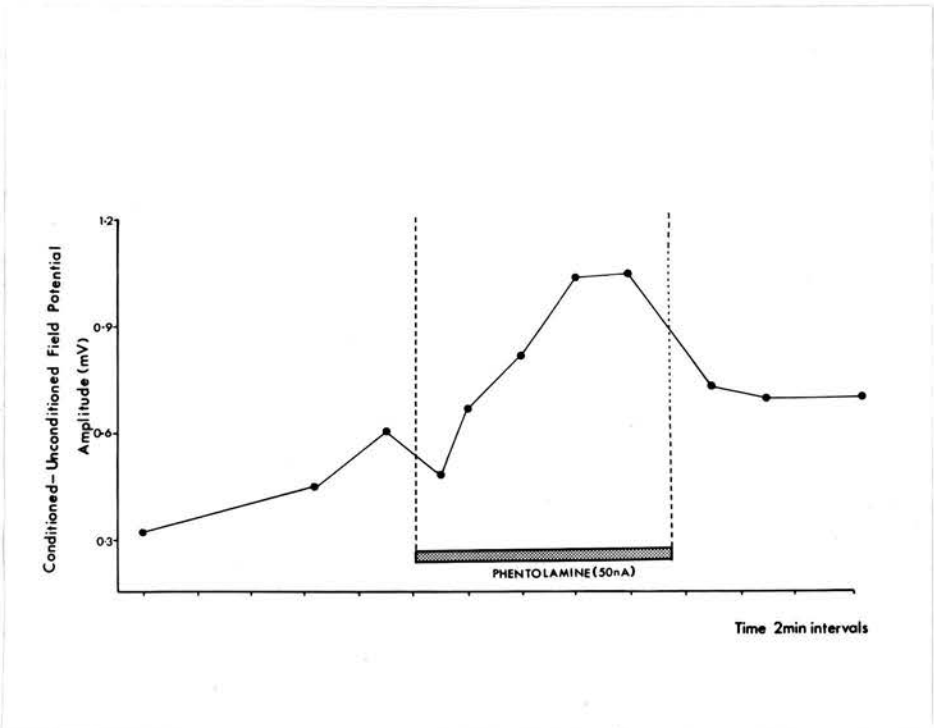

FIGURE 40

The effect of iontophoretically applied phentolamine on the difference between the conditioned and unconditioned orthodromically evoked field potential. The period of application is indicated by the bar along the abscissa.

Cinanserin

Cinanserin has been iontophoretically applied in three separate recording situations in 2 animals. On each occasion the antagonist reduced the amplitude of the conditioned field potential. The time course was similar to that recorded for the orthodromic field potential.

Effects of other iontophoretically applied agents

A noradrenergic α-receptor blocking agent has been iontophoretically applied during the recording of the orthodromic field potential. In this particular recording situation 5-HT increased and cinanserin reduced the amplitude of the conditioned field potential.

Fig.40 illustrates the effect of 50 nA. of phentolamine applied over a period of 10 minutes. The effect of the drug was to increase the difference in amplitude between the conditioned and unconditioned field potentials. The drug response became maximal 6 minutes after the application had commenced and began declining about 2 minutes later. There was no evidence of a blocking of the potentiating effects of midbrain stimulation. Thus the effects of phentolamine contrasted with those of the serotonin antagonists.

D I S C U S S I O N

DISCUSSION

The aim of these experiments has been to examine the possibility that 5-HT may have a neurotransmitter role at lumbar spinal motoneurones. Three distinct experimental procedures have been employed, each designed to monitor changes in excitability of motoneurones resulting from evoked activity in the descending 5-HT containing pathway. To identify the involvement of 5-HT in this change, attempts have been made to interfere with the effect by changing 5-HT receptor sensitivity and by altering 5-HT metabolism.

Intracellular Recordings

Although the rat has not traditionally been employed in experiments involving a laminectomy, the present series suggests that it is quite suitable for such procedures. Experiments demanding either long term extracellular recordings or prolonged MSR measurements have been successfully accomplished using rats. The limitations of the rat as an experimental animal became evident during the intracellular recording study. Many more intracellular recordings per animal have been made in cat than in rat. This partly reflects the decision to use rats in the preliminary experiments in which microelectrode techniques were still being developed. However, it also provides an indication of the greater stamina under experimental conditions of the cat compared to the rat.

The relative mechanical instability of the rat spinal cord which accounted for some of the problems associated with the intracellular parallel microelectrode study, was partially overcome by recording from cat motoneurones. The

extra stability provided by the cat was found to be essential when using this type of electrode. In addition the experimental cats often remained healthy for 18 hours of experimentation (compared to about 9 hours for a rat).

In the experiments during which intracellular recordings have been obtained from both cat and rat motoneurones, slight differences were observed between the antidromic spike configuration recorded from the two types of neurone. These differences confirm the earlier observations of Bradley and Somjen (1961).

The slightly larger and longer hyperpolarisation recorded from cat motoneurones may be associated with the tendency of rat motoneurones to fire at a rate faster than that usually found in cat motoneurones,.(Bradley and Somjen 1961). It has been suggested that a phase of delayed depolarisation may mask the presence of the after-hyperpolarisation in rat motoneurones (Granit, Kernell and Smith, 1963). These authors suggest that the basis of the effect is an invasion of the dendrites by the action potential. Thus differences in the cytoarchitecture of rat and cat motoneurones may, it is argued, be responsible for the differences in antidromic spike configuration.

Alternatively these differences may be associated with differences in the average diameter of cat and rat motoneurones. Although no obvious difference between cat and rat motoneurone membrane potentials have been recorded, it seems possible that the smaller rat motoneurone may suffer proportionally more damage during impalement by the recording electrode, than the larger cat motoneurone.

Stimulation of the brain stem in the region of the raphe nuclei elicited complex intracellular responses in lumbar motoneurones. The early membrane response corresponding to EPSPs had a latency of between 1.5 and 2.5 msecs. Assuming a monosynaptic pathway of about 10 cms in rat the conduction velocity of the pathway mediating the response is between 40 and 60 metres/sec. However the conduction velocity of a 5-HT pathway in the brain stem has been tentatively calculated from experimental data to be between 0.6 and 0.8 metres/sec. (Couch 1970), this corresponds well with the observed small diameter (1-2 μ) of these unmyelinated fibres (Dahlstrom and Fuxe, 1965 II).

In view of the calculated conduction velocity and the reported pharmacology of the pathway mediating the short latency EPSPs, (see later in Discussion) it seems unlikely that a 5-HT containing pathway is involved in the transmission of such responses.

Having reduced the possibility of the involvement of a monoamine pathway in the short latency response, it is of interest to know which descending pathway could be mediating the response. One may speculate that the most appropriate fast-conducting pathway appears to be one descending in the medial longitudinal fasicle and originating from the nucleus reticularis pontis caudalis (Grillner, Hongo and Lund (1971). This nucleus is located in a similar anterior-posterior plane as that of the raphe nuclei and 1.5 mm. lateral to it. The possibility cannot be excluded that current spread from the raphe stimulating electrode was responsible for electrical

stimulation of this lateral nucleus. Some support for this explanation is provided by differential stimulation experiments. During stimulation in the coronal plane it was observed that stimulation of lateral regions was more successful in eliciting EPSPs than stimulating in the midline. The observation that stimulus currents of 300 μA (monopolar stimulation) often co-activates fibres running 2-4 mm. lateral to the point of stimulation (Grillner et al., 1971) lends further support to this hypothesis.

The origin of the long latency, long duration response will now be discussed. Both the latency of the response and the fact that the response was preferentially elicited from the midline of the brain stem suggest that the membrane response could have been due to activity in fine unmyelinated fibres originating from the raphe nuclei. There are however several alternative explanations which may be considered. The prolonged intracellular response could have been due to activity in spinal polysynaptic pathways. Alternatively, stimulation in the brain stem may have activated supraspinal structures ultimately responsible for modifying motoneurone excitability.

In an attempt to assess the possible involvement of the forebrain region in the mediation of raphe stimulation, a series of experiments was performed with decerebrate rats. These experiments indicate quite clearly that changes in motoneurone excitability resulting from raphe stimulation are not necessarily mediated by fore brain structures.

The involvement of spinal polysnaptic pathways is

discussed later, but it seems unlikely that the prolonged intracellular response is due to such activity, since results from the MSR study indicate that raphe stimulation appears to have opposite effects on polysynaptic and motoneurone excitatory states.

From these considerations alone, it appears that the long duration intracellular response may indeed result from activity in the raphe-spinal pathway. Further evidence was provided by the pharmacological study.

In an attempt to study the pharmacological basis of the postsynaptic membrane responses, drugs having 5-HT receptor blocking properties were iontophoretically applied to the vicinity of lumbar motoneurones. The technique of intracellular recording coupled with extracellular iontophoresis is technically difficult. Although intracellular recordings using single recording electrodes are not difficult to obtain, the instability associated with parallel microelectrodes frequently resulted in the loss of membrane potential. Of the 15 motoneurones studied only 6 showed any evidence of a response modification after the iontophoretic application of antagonists.

The absence of drug effects may be attributed to several factors. These include a) absence of 5-HT receptor sites, b) iontophoretically applied drug not reaching the receptors, c) result of the drug-receptor interaction not affecting the somatically recorded membrane potential and d) fluctuations in the membrane potential masking the effect of drug-receptor interaction.

If, as seems likely many 5-HT receptor sites are located on dendrites (Dahlstrom and Fuxe, 1965), it is possible that the exogenously applied drug may never reach the target site. It must be remembered that a dendritic tree can extend to as much as 2 mm. from the motoneurone soma (Aitken and Bridger, 1961).

Although the theoretical aspect of the iontophoretic release of substances has been examined in detail (Curtis, Perrin and Watkins, 1959), in vivo studies designed to identify the extent of diffusional pathways of iontophoretically released agents have yielded little information (Herz, Zeiglgansberger and Farber, 1969). However it seems unlikely that target sites more than 200-300 μ from the pipette tip will be affected by the released agent. In addition the active uptake processes probably reduce the extracellular concentration of the exogenously released drug.

Thus if many receptors are located on dendrites of motoneurones, it seems very unlikely that sufficient quantities of iontophoretically applied drugs will reach the receptor sites. This is especially relevant when it is appreciated that the tip of the parallel iontophoresis pipette is very close (30 μ) to the soma. It is interesting to note that during the extracellular iontophoresis study, a period of between 3 and 10 minutes was often required before drug effects became evident. Intracellular recordings accompanying iontophoresis studies rarely provided stable membrane potentials for these lengths of time. It seems likely therefore that the period of iontophoretic application during

the intracellular studies was insufficient.

Receptor sites within the sphere of influence of the exogenously released drug will presumably interact with the drug and produce a local change in membrane potential. However the existence of a somatically recorded effect is questionable. Although it has been suggested that 'synapses on dendrites are virtually ineffective if situated on the more remote regions of dendrites' (Eccles, 1957), even the more distally located synapses play some part in controlling motoneurone excitability.

However a somatically located electrode may well be incapable of discerning small changes in membrane potential resulting from the interaction between iontophoretically applied drug and the necessarily small proportion of affected receptor sites.

If the manifestation of the drug-receptor interaction is recorded from the motoneurone soma, presumably such activity can only be monitored in a cell having an extremely stable membrane potential. This is because a careful examination of the relationship between membrane potential and intracellular response amplitude has indicated that a small reduction in membrane potential produces a disproportionately large reduction in response amplitude. The relative instability of the membrane potential during parallel microelectrode studies may well account for the inconsistent drug effects.

On those occasions when the membrane potential was particularly stable throughout the local application of a 5-HT antagonist, it is apparent that there has been some

attenuation of the long duration response amplitude.

The above considerations go some way to interpreting the rather inconsistent results obtained from the parallel microelectrode study. Although the technique is a very powerful one, especially when confirming the identity of a putative neurotransmitter, its application in the resported project is limited. From the limited results provided by the study it does seem that there are 5-HT receptors close to lumbar motoneurones.

Further support for this theory was provided by the study involving the systemic administration of 5-HT antagonists whilst recording intracellularly. The greater degree of membrane potential stability associated with this study is accounted for by the use of the smaller single recording electrodes. Despite this greater stability, a gradual loss in membrane potential often accounted for a subsequent loss of response amplitude. There were however studies in which there appeared to be an authentic pharmacological effect. Of the 10 cells studied in this way there was little evidence of any consistent attenuation of the membrane responses. On those occasions in which there appeared to be a reduction of the responses, careful examination of the membrane potential indicated a small loss in potential. Such a reduction in membrane potential was probably sufficient to account for the reduced response amplitude.

Recording intracellular responses from a group of cells to raphe stimulation before and after injection of LSD, is unlikely to have identified a limited pharmacological effect,

it would however have identified any substantial blockade of the response. That such an effect has not been identified suggests that the intracellularly recorded response may not be entirely effected via a 5-HT pathway. Alternatively the absence of an effect may result from the failure to administer a sufficiently large dose of the antagonist. However since the maximum dose of applied LSD was far in excess of that observed in the MSR experiments to block 5-HT effects, this seems unlikely. The predominance of excitatory effects of supraspinal stimulation on motoneurone excitability reported here is interesting. Although IPSPs have been recorded on occasions, they were never recorded with any regularity. The relative absence of such potentials may be either because the stimulated pathways were predominantly excitatory or possibly because inhibitory components are being suppressed.

In a recent study Frank and Ohta (1971) have demonstrated that some anaesthetic agents (both barbiturate and non-barbiturate) are capable of preferentially blocking reticulospinal inhibitory influences on spinal reflex potentials. Unfortunately these authors did not examine the effects of halothane anaesthetics on descending influences. However since predominantly excitatory effects were observed in the reported experiments even in the absence of anaesthetic (i.e. in decerebrate preparations), the possiblity that the anaesthetic abolished inhibitory effects is small.

Although it thus seems probable that the stimulated pathway was predominantly excitatory, the only other published

attempt to stimulate the raphe-spinal pathway produced rather different results. Clineschmidt and Anderson (1970) reported that if an MSR was evoked 2.5 msec. after the end of the conditioning stimulation, potentiation of the MSR was more frequently observed, but if the interval was increased to 25 msec, inhibition was most frequently observed. Subsequently these authors noted that only the inhibitory effects were blocked by (large) intravenous doses of LSD.

In the present series of experiments, excitatory effects of raphe nuclei stimulation were observed up to a condition-dorsal root stimulus interval of 70-80 msec. Longer stimulus intervals were associated with inhibitory effects (see Fig.25). In contrast to Clineschmidt and Anderson the present findings indicate that the excitatory effects were blocked by small intravenous injections of three different 5-HT antagonists.

The similarity between the study reported here and that of Clineschmidt and Anderson (1970) lies in the common observation that shorter condition-test intervals were associated with potentiating effects whilst longer intervals resulted in inhibition of reflex activity. Possibly the later inhibitory influence results from activity in spinal interneuronal pathways. If the relevant spinal interneuronal system were inhibitory, its effect on motoneurone excitability may only become evident after an interval of many milliseconds. Although speculative in nature, such a theory is supported by the observed reduction in PSR activity after stimulation of the raphe nuclei (see Results and later in Discussion

where it is suggested that activity in the raphe-spinal pathway may excite inhibitory spinal interneuronal pathways).

Motoneurone excitability recorded from spinal ventral root

Changes in MSR amplitude provide. an index of motoneurone excitability just as monitoring changes in membrane potential indicated changes in excitability of single motoneurones during the intracellular study. Similarly, recording spontaneous activity from the ventral roots also reflected the level of excitability of the motoneurone pool.

The spontaneous activity recorded from the ventral root was of course action potenials generated in motoneurones and propagated down the ventral roots. Reference has been made to experimentally quiescent motoneurones, the activity recorded during this study indicates that motoneurones may be spontaneously active provided the level of anaesthesia is sufficiently low. Although the level of applied anaesthetic was constant, small apparently spontaneous fluctuations in the depth of anaesthesia frequently limited the study of the spontaneous motoneurone discharge.

However 6 animals were studied in which the pharmacology of the discharge in response to the stimulation of raphe nuclei was examined. Stimulation of the raphe nuclei over a period of 10 seconds increased the number of action potentials recorded from the ventral root over a period of up to 60 seconds. This prolonged discharge was almost totally abolished within 60 seconds of the intravenous application

of LSD (10 µg/kg).

The dramatic blocking of the evoked discharge immediately suggested the involvement of 5-HT in the evoked activity. This study provided the first firm evidence that 5-HT may be involved in the modification of motoneurone excitability. Furthermore the study also suggested that stimulation of the raphe-spinal pathway results in an increase of motoneurone excitability.

Unfortunately small spontaneous changes in the depth of anaesthesia frequently rendered the spontaneous motoneurone discharge difficult to study. This effect also probably accounted for the 10% variation occasionally recorded in successive monosynaptic reflex amplitudes.

On the other hand this variation has not interfered with the quantitative analysis of experiments involving recording MSR amplitude in one particular rat, over periods of several hours. Thus the controlled delivery of Fluothane has enabled a level of anaesthesia to be maintained which was sufficient to allow MSRs of constant amplitude to be recorded over periods of 4 hours or more.

Unfortunately the stability of the MSR evoked by dorsal root stimulation did not extend to the PSR. Activity in spinal interneuronal pathways appears to be more susceptible to the gradual deterioration of the preparation.

Spinal motoneurone activity and 5-HT

The monosynaptic reflex recorded from the ventral root showed a sigmoid increase in amplitude with increasing dorsal root stimulation. Although the exact configuration varied

from one rat to another, several general features of the curve have always been observed (see Fig. 24 and 28).

As indicated above, the amplitude of the monosynaptic reflex provides some measure of the proportion of the available lumbar motoneurones generating action potentials in response to dorsal root stimulation. At low stimulus intensities few motoneurones are active and the MSR amplitude is small. At intensities of stimulation corresponding to medium sized MSR amplitudes, a small change in stimulus intensity elicits a considerable change in MSR amplitude. The sensitivity of the MSR amplitude over this restricted range of stimulus intensities can probably be explained by the existence of a subliminal component of the motoneurone pool. Neurones represented by this component are in a state of semi-excitation i.e. although close to generating an action potential the dorsal root activity is just insufficent to cause the neurone to fire. A slight increase in dorsal root activity will then elicit action potentials from a hign proportion of this sub-liminal component.

At higher dorsal root stimulus intensities almost all motoneurones capable of responding are active, a further increase in stimulus intensity can only marginally increase the number of active neurones. This situation is reflected by the upper part of the sigmoid curve, where increasing the dorsal root stimulus intensity elicited only a small increase in MSR amplitude.

These stimulus-response curves have been constructed to examine the effects of conditioning stimulation and drugs

on motoneurone excitability. If the effect of these variables had been examined only at supramaximal MSR amplitude, it seems possible that any excitatory effects may have been masked by the inability of the system to respond. Thus if 100% of available motoneurones are responding to dorsal root stimulation an increase in motoneurone excitability resulting for example from the effects of conditioning stimulation, may not be apparent.

In practice, the extent of conditioning potentiation of the MSR has been shown to be relatively independent of the dorsal root stimulus intensity. Even at dorsal root stimulus intensities corresponding to a maximal MSR, conditioning stimulation increased MSR amplitude. Conditioning stimulation of the raphe nuclei, is then presumably capable of exciting a group of motoneurones which are not normally 'activated' by stimulation of a particular dorsal root. The size of this secondary pool is constant, since as indicated above, the extent of the conditioning effect is constant at almost all dorsal root stimulus intersities (and hence MSR amplitudes).

By varying the time interval between the end of the conditioning stimulation and the elicitation of the MSR, it has been demonstrated that the period of maximum potentiation coincides with the period immediately following conditioning stimulation.

The extension of the interval to between 20 and 30 msecs. results in a reduction of the potentiating effects of conditioning stimulation. In an attempt to relate the intra-

cellular motoneurone responses to raphe stimulation to the MSR potentiating effects, the time course of the two effects has been compared. It is apparent that the slow depolarisation response immediately following the conditioning train represents a period of increased motoneurone excitability which coincides approximately with the interval following conditioning stimulation in which the MSR is potentiated. The experimental results do not positively confirm or deny the possiblity that the slow response is the membrane effect underlying the increase in motoneurone excitability reflected by the potentiated MSR. However, since the pharmacology of both the slow response and the MSR potentiation (see later) seem to have a common basis, it seems possible that they are both manifestations of activity in a common descending pathway.

A direct relationship between the raphe nuclei and changes in motoneurone excitability has also been demonstrated by differentially stimulating brain stem areas. The results of these studies suggest that both the slow depolarising response and the MSR potentiating effect are preferentially elicited from the midline of the brain stem. That the effects were also on occasions elicited when the stimulating electrode was placed slightly lateral to the midline may be explained in terms of the spread of stimulating current. Biochemical studies (Shields and Eccleston 1972) involving the electrical stimulation of the raphe nuclei via stimulating electrodes, indicates that the precise electrode position is very important when considering changed levels of metabolites.

A further difference between the present results and the biochemical study of Shields and Eccleston (1972) relates to the optimum stimulation frequency. Of course, the use of the term 'optimum' in this sense can be misleading, the optimum frequency in an experimental situation refers to the frequency evoking the largest change in the system being monitored. Shields and Eccleston observed the biggest modification of 5-HT metabolism when the stimulation frequency was set at 10 Hz. In the particular system being monitored in the present study, motoneurone excitability, stimulation at frequencies below 60 Hz failed to modify excitability. An important difference between the studies is that in the present series of experiments, stimulation was phasic in nature, whereas in the biochemical study, the raphe nuclei were stimulated tonically for periods of one hour.

Although stimulation in the region of the raphe nuclei was responsible for i) a depolarising membrane response and ii) a potentiation of spinal MSR, the involvement of 5-HT in the mediation of the former effect has not been completely established. However, experiments designed to assess the extent to which MSR potentiation was due to transmission in a 5-HT pathway have indicated that a considerable proportion of the effect can be attributed to such activity.

All three 5-HT antagonists investigated demonstrated some blocking of the potentiating effect. Higher doses of the antagonists also reduce the amplitude of the unconditioned MSR. Banna and Anderson (1968) argued that one of the 5-HT antagonists, methysergide, may have a depressant effect on

motoneurone excitability via an action other than the antagonism of 5-HT. These authors demonstrated that in a 5-HT depleted preparation, methysergide was still capable of depressing motoneurone excitability. The endogenous 5-HT had been depleted by chronic cord transection and pretreatment with reserpine. However unless it can be experimentally verified that all neuronal 5-HT has been eliminated (and these authors admit that not all 5-HT was depleted) it is still possible that the remaining low levels of 5-HT may be effective in modifying motoneurone excitability. The depressant action of methysergide is then explicable in terms of its effect on 5-HT receptors. Thus in the present experiments the reduction in the unconditioned MSR amplitude can also be explained in terms of a pharmacological antagonism. If there were some tonic activity in the raphe-spinal pathway in the absence of raphe stimulation, then the reduction of the unconditioned MSR amplitude may be the result of the pharmacological antagonism of this activity.

The potent blocking effects of extremely small doses of LSD on reflex activity reported here, contrast with earlier reports (Geiger and Cervoni, 1958; Banna and Anderson, 1968; Clineschmidt and Anderson, 1970). It may be of significance to note that in the present study maximum dose levels of LSD were about 10 µg/kg. In the reports cited above, doses below 200 µg/kg had no effect on spinal reflex activity and maximum dose levels were in the region of 0.5-1.5 mg/kg. In a recent review, Aghajanian (1972) has noted the marked differences in the effect of LSD in various situations and suggests that

variations in experimental conditions (e.g. anaesthesia, dosage, route of administration) may account for some of these anomalies.

Knowledge concerning the mechanism of action of LSD is still so vague that few conclusions can yet be reached. Although it seems clear that LSD can interfere with the pharmacology of 5-HT, whether it mimics or antagonises the actions of the monoamine seems to depend upon experimental factors. For example, Banna and Anderson (1968) observed that LSD (300 µg/kg) increased MSR amplitude in the acute spinal cat, yet in the same preparation other drugs noted for their 5-HT antagonistic activity (methysergide and 2-brom-LSD) reduced MSR amplitudes. Furthermore during these experiments LSD (1.5 mg/kg) reduced the facilitatory effects of 5-HTP and L-tryptophan on the MSR, possibly via a pharmacological antagonism of 5-HT.

The results of the present study suggest that extremely small doses of LSD can block the excitatory effects of 5-HT on motoneurone excitability. It is interesing to note that similar doses of intravenously administered LSD have also been reported to produce a reversible cessation of the firing of single neurones in the raphe nuclei, (Aghajanian, Foot and Sheard, 1969). The latter effect can either be interpreted as an antagonism or agonism since both excitatory (Couch 1970) and inhibitory (Aghajanian, Haigler and Bloom, 1972) effects of iontophoretically applied 5-HT have been observed on raphe neurones.

The consistent reduction of the excitatory effects of

conditioning stimulation and L-tryptophan on reflex activity by all three 5-HT antagonists in the present study seems to confirm a) the involvement of 5-HT in the effect and b) that LSD in small doses can antagonise the excitatory actions of 5-HT.

Because of the uncertainty of the action of the larger doses of pharmacological antagonists, great care has to be taken to use the smallest effective dose. In most instances the dose of antagonist chosen was sufficiently small not to affect the unconditioned MSR.

All three 5-HT antagonists were extremely effective in reducing the potentiating effects of conditioning stimulation. The most effective antagonist appears to be LSD. Between 5-10 µg/kg LSD reduced the potentiated (conditioned) MSR by 75% in three animals. In other preparations the potentiation was only reduced by 33%. These differences may possibly be explained by a) the extent to which the effects of conditioning stimulation are being mediated via 5-HT mechanisms rather than other systems or b) the capacity of the lumbar motoneurone pool to respond to the descending 'serotonergic' influence. The effects of these variables are discussed below.

If 75% of MSR potentiation is blocked by a 5-HT antagonist, the remaining 25% may either have a non-serotonergic basis and thus will continue to resist even the blocking effect of higher doses of 5-HT antagonists, or is serotonergic, but in the absence of higher concentrations of the antagonist, will not be blocked. Increasing the dose of antagonist does

frequently further reduce the conditoned MSR, unfortunately at these higher dose levels, the unconditioned MSR is also reduced in amplitude although this may be due to the specific antagonism of 5-HT receptors activated by spontaneous activity in the raphe-spinal pathway, it may also be due to a non-specific effect on non-tryptaminergic mechanisms. It therefore seemed preferable to study doses of LSD which were more likely to be exerting specific effects.

It must be recognised that the change in amplitude of the MSR due to stimulation in the brain stem may well be the integrated response of the motoneurone pool to both descending facilitatory and inhibitory influences. If as seems likely, the excitatory component is largely mediated via a 5-HT system, the removal or blocking of this component may unmask the inhibitory influence. The result of this interpretation would be that the reduction in the conditioned MSR amplitude by 5-HT antagonists is partly due to a real pharmacological effect and partly due to activity in an inhibitory pathway. This interpretation is purely speculative and although there is no evidence either to support or deny the theory, the existence of a descending inhibitory component should be considered.

A second possible explanation for the variability of the blockade by 5-HT antagonists is based upon the distribution of 5-HT within the spinal cord. Recording the MSR from motoneurone axons has provided a monitor of the excitability of a restricted sample of spinal motoneurones. Fluorescence histochemistry studies have demonstrated the presence of 5-HT containing terminals close to neurones in the lumbar cord.

There are however no published reports indicating the exact distribution of the monoamine along the extent of the lumbar enlargement. It is apparent that there is a higher concentration of 5-HT within the enlargement than at the anterior and posterior borders. It could be argued that since the distribution of motoneurones follows a similar pattern, the innervation ratio (i.e. number of 5-HT terminals: number of motoneurones), is constant. However it seems unlikely that neighbouring ventral roots originating from different regions of the lumbar enlargement will have exactly comparable innervation ratios. If this is the case, then conditioning stimulation applied to the raphe nuclei will necessarily affect reflexes evoked from neighbouring ventral roots, to different extents. It may then be this variation which accounts for both the different degree of MSR potentiation and the varying degree of blockade by antagonists.

It might be argued that the inevitable progressive deterioration of the animal during the experiment might account for the observed reduction in MSR potentiation after the administration of 5-HT antagonists. Had this been the case the unconditioned MSRs would also have been reduced and they were not. Thus the unconditioned MSR can be regarded as a control. A much more positive denial of this arguement is provided by the fact that the full recovery of the potentiating effect was frequently observed. The 'rebound' increase in excitability recorded after the reattainment of predrug MSR amplitudes is difficult to explain. Not only did the unconditioned MSR increase, but so did the descending potentiating

effect.

The modification of the MSR potentiation by 5-HT antagonists confirms and quantitatively extends the earlier result from the study of spontaneous activity recorded in the ventral root. Stimulation of the raphe nuclei leads to an increase in excitability of motoneurones, the pharmacology of the effect involving 5-HT at some point.

Experiments involving the administration of L-tryptophan were an extension of earlier studies by other workers (Anderson and Shibuya, 1965; Shibuya and Anderson, 1968; Marley and Vane, 1967). The present results confirm their earlier observations that L-tryptophan increases the amplitude of the MSR. Furthermore, the present series of experiments demonstrates that the precursor induces a disproportionately greater increase in the conditioned reflex when compared with its effect on the unconditioned reflex. This result provides further evidence indicating that the effect of raphe stimulation on the spinal MSR is mediated via a serotonergic system.

The slow development of the change in motoneurone excitability is consistent with the postulate that it is due to a metabolic product of L-tryptophan. The most likely product is 5-HT since previous studies have reported that L-tryptophan elevates 5-HT levels in brain (Bogdanski, Weissbach and Udenfriend, 1958; Weber and Horita, 1965) and spinal cord (Anderson and Shibuya, 1966).

Possibly this elevated level of CNS 5-HT increases the tonic activation of tryptaminergic receptors, some of which may be on lumbar motoneurones. An increased level of

tonic activation of these receptors would account for the observed increase in the unconditioned MSR after treatment with L-tryptophan. Although it may be argued that the increase in excitability could be due to metabolic products of L-tryptophan other than 5-HT, the blocking of the effect by 5-HT antagonists strongly denies this possibility.

It has been postulated that 5-HTP and L-tryptophan cause an excessive production of 5-HT in the terminals of 5-HT containing neurones (Anderson & Shibuya 1966). In view of fluorescent histochemistry studies before and after administration of 5-HT precursors (Fuxe 1965), it seems likely that the above interpretation is correct. The abnormally high levels of 5-HT in the presynaptic terminals would then account for the greater efficacy of raphe stimulation. The serotonergic basis of the effect is further demonstrated by the total blocking of precursor-induced increase by 5-HT antagonists.

The increased MSR amplitude following conditioning stimulation and L-tryptophan together with the reduction in amplitude following the administration of LSD, reflect changes in the excitability of motoneurones. These changes in excitability could result from a) changes in the effectiveness of afferent (dorsal root) stimulation, b) a tonic increase or decrease of spinal interneuronal pathways (i.e. indirect modification) or c) a direct modification of motoneurone excitability.

Since dorsal root afferent fibre terminals do not contain 5-HT, it seems unlikely that agents which modify

neuronal 5-HT levels can change the efficacy of afferent stimuli. Furthermore the effects of conditioning stimulation on the MSR (an effect not involving transmission in dorsal root afferents) can be blocked by 5-HT antagonists at a time when unconditioned MSR remains unaffected by the antagonist.

Evaluation of the involvement of spinal interneurones in the activity of the serotonergic system is difficult. The results obtained however, clearly indicate differences in response by motoneurones and interneurones to conditioning stimulation. Stimulation of the raphe nuclei increased the amplitude of the MSR whilst reducing PSR amplitude (see Fig.23 and later in Discussion). It seems unlikely therefore that the increase in motoneurone excitability resulting from conditioning stimulation could be due to an increase in tonic interneuronal excitability.

These considerations support the postulate that increased MSR amplitude in the above situations is achieved via a direct increase in motoneurone excitability. Further support for this postulate has been provided by the results of the iontophoresis experiments. The response of motoneurones to the local application of drugs which mimic or block the actions of 5-HT, strongly suggests that the 5-HT containing terminals that have been identified close to motoneurones are concerned with the control of motoneurone excitability.

Motoneurone field potentials

An examination of the observed changes in extra-

cellularly recorded field potentials during the iontophoretic application of drugs has led to the formulation of two theories concerning the mode and site of action of 5-HT. The two theories hinge on the interpretation of the orthodromically evoked field potential. It is postulated that the field potential may either predominantly reflect i) activity of a group of neurones in the motoneurone pool, ii) activity in a few local dendrites close to the recording electrode or iii) activity in both dendrites and motoneurone somata. These possibilities will be discussed below.

If it is assumed that the field potential does reflect activity in local dendritic elements, then an increase in the field potential suggests an increase in the number of local dendrites conducting action potentials. Part of the complex field potential elicited by stimulation of the dorsal roots may represent the antidromic invasion of dendrites by the orthodromic action potential generated in the motoneurone soma.

Preceding the dorsal root volley by conditioning stimulation, markedly increases the amplitude of the field potential. This may be explained in the following manner. Conditioning stimulation applied to the raphe nuclei results in the release of 5-HT from synaptic terminals situated on dendrites. The release of 5-HT depolarises the dendrites to a limited extent. This moderate depolarisation is sufficient to enable the antidromic invasion of those dendritic elements that before had resisted the conduction of the potential. It is this increase in the number of

'active' dendritic elements that is reflected in the increased field potential amplitude.

Although there is evidence to suggest that dendritic impulse propagation occurs in spinal motoneurones (Fatt 1957; Terzulo & Araki 1961), in Purkinje cells at least the conduction is all-or-nothing in the proximal dendrites and becomes decremental as it approaches the terminal branches (Eccles, Llinus & Sasaki 1966). Thus a small depolarisation of a proximal dendrite might enable an action potential to be propagated along the dendrite whilst in a distal dendrite the same depolarisation would reduce the rate of decrement of the signal.

If conditioning stimulation does have its effect on the motoneurone via the release of 5-HT, then the local exogenous application of 5-HT would be expected to behave in a similar manner. The inconsistent effect of endogenous 5-HT on the unconditioned orthodromic field potential however is difficult to explain. Possibly insufficient amounts of the amine reached the receptor sites, thus the effect of the drug is insufficient to cause any change in the field potential.

The increase in the conditioned orthodromic potential subsequent to the exogenous application of 5-HT can also be explained in terms of an action on dendrites. The additional increase in the field potential amplitude indicates a further increase in excitability of the neuronal system. Possibly the effect of 5-HT, as with conditioning stimulation, was to increase the antidromic invasion of

the dendrites by the somatic spike.

Although an explanation of the observed effects of both conditioning stimulation and drugs can be discussed in terms of a dendritic action, it seems possible that field potentials associated with randomly arranged dendrites may well cancel out. If this is the case, the recorded potential could not then represent activity in dendritic elements.

An alternative interpretation of the field potential would be that the microelectrode records the activity of many motoneurones simultaneously. The number of motoneurones firing in response to stimulation of the dorsal root is reflected by the amplitude of the field potential. If stimulation of the raphe nuclei increases the total number of motoneurones depolarising, the field potential amplitude should increase - as it does. Excitability of motoneurones is thus assessed by the change in field potential amplitude just as the change in MSR amplitude recorded from ventral roots reflects differences in motoneurone excitability. The increase in field potential amplitude resulting from the iontophoretic application of 5-HT would then be explained in terms of an increase in the number of motoneurones depolarising in response to dorsal root stimulation.

The limited sphere of influence of iontophoretically applied drugs may seem to detract from the validity of the alternative explanation. Previous workers (Herz, Zieglgangsberger & Faber 1963) have demonstrated that iontophoretically applied drugs only affect structures

within 200-300 μ of the micropipette tip. However since dendrites from several motoneurones probably exist within this sphere of influence, iontophoretically applied drugs may well affect activity in dendrites which subsequently modify motoneurone activity.

The interpretation of field potentials in terms of dendritic effects has been recently reported. Ramos (1971) has suggested that mescaline may act by increasing the responsiveness of cortical dendrites via a small dendritic depolarisation. In addition, Bloom et al. (1970) have demonstrated that noradrenaline-containing terminals make contact with the major Purkinje dendrites and small dendritic spines.

Although field potentials have been discussed above either in terms of a somatic or dendritic origin, such an interpretation is probably somewhat naive. Since both the dendrites and somata are part of the same electrical system (i.e. a neurone) their respective activities can never be totally independent. For example, uninvaded regions of dendrites (uninvaded, that is , by an action potential) act as a source for flow of current into the invaded parts (Barakan, Downman & Eccles 1949). It seems likely therefore that the field potentials recorded during the present study reflect activity in both dendrites and somata.

In the present study the iontophoretic application of cinanserin has been observed to a) block the potentiating effects of conditioning stimulation on the orthodromic field potential and b) reduce the facilitatory effects of

locally applied 5-HT on the same field potential (Fig.39). The reduction of the conditioned field potential by a 5-HT antagonist provides further evidence that 5-HT is involved in the control of motoneurone excitability. More significantly this observation provides the first evidence that the increase in motoneurone excitability associated with stimulation of the raphe-spinal pathway involves the action of 5-HT close to the motoneurone.

It could be argued that the excitatory effects of conditioning stimulation and the local application of 5-HT and 5-HT antagonists may all result from the inhibition by 5-HT of an inhibitory interneurone close to the motoneuorne. In fact such an explanation has been postulated by Clineschmidt & Anderson (1970). However the existence of 5-HT terminals close to motoneurones and the absence of a 5-HT-containing interneurone (see later) (Dahlstrom & Fuxe 1965), suggests that the direct effect is more likely.

Although at this stage no other 5-HT antagonist has been applied, phentolamine -- an α-adrenergic receptor blocking agent - has been iontophoretically applied (see Fig.40). The control antagonist did not decrease tha amplitude of the potential. In fact the observed increase in the conditioned reflex may be explained in terms of the blocking of a tonic inhibitory influence. This observation supports earlier reports of the inhibitory effects of exogenously applied noradrenaline on spinal motoneuornes (Engberg & Ryall 1965). Further evidence for an inhibitory role for noradrenaline at spinal motoneurones has been provided by Weight &

Salmoiraghi (1967). These authors using a similar technique to the one reported here, iontophoretically applied noradrenaline to the synaptically (orthodromic) evoked field potential. The potential became attenuated, suggesting decrease in motoneurone excitability.

If the theory of a direct excitatory action of 5-HT on lumbar motoneurones is accepted, earlier models conceived by other workers to explain their observations will have to be rejected.

The results presented here are in direct conflict with the only other reported attempt to electrically stimulate the raphe-spinal pathway. As already discussed, Clineschmidt & Anderson (1970) found that although both excitatory and inhibitory effects on the spinal MSR could be elicited by stimulation of the raphe nuclei in cat, only inhibitory effects were blocked by 5-HT antagonists. Furthermore, the relative insensitivity of reflex activity to LSD as reported by the above authors is difficult to explain.

To account for these and other observations concerning the estimated conduction velocity of the raphe-spinal pathway, Clineschmidt & Anderson (1970) postulated the existence of an inhibitory 5-HT containing interneurone in the spinal cord. The evidence for such an interneurone is meagre. The authors argue that the reported levels of 5-HT in the cord 3 weeks after a transection of the spinal cord (Shibuya & Anderson 1968) indicate the possible existence of a 5-HT containing interneurone. However the technique

of fluorescence histochemistry is far more sensitive than the biochemical techniques and results from many studies (Dahlstrom & Fuxe 1965; Fuxe 1965) deny the existence of 5-HT containing cells outside the brain stem region.

Although 5-HT has been shown to elicit an increase in excitability of spinal motoneurones a discussion of a neurotransmitter role for 5-HT must be approached with caution. The present study has demonstrated that stimulation of the raphe-spinal pathway results in an increase in motoneurone excitability. The increase in excitability appears to be accomplished via an action of 5-HT close to the motoneurone.

It could be argued that the increase in field potential amplitude resulting from the iontophoretic application of 5-HT is due to the stimulation of specific chemosensitive areas of the neurone rather than subsynaptic sites, as there is evidence that at least in non-vertebrate systems two distinct sites exist (Kerkut & Walker 1961; Gerschenfeld & Tauc 1964). However the blocking effect of exogenously applied cinanserin suggests that stimulation of the raphe-spinal pathway does in fact release 5-HT onto a post-synaptic membrane.

The unsatisfactory nature of the intracellular recording study means that little data concerning the post-synaptic ionic processes accompanying the postulated release of 5-HT has been gained.

In the absence of information concerning the equilibrium potential for both the physiological synaptic

transmitter (stimulated pathway) process and for the exogenously applied 5-HT it is not possible to discuss the possible similarities in ionic action for the two substances.

The equilibrium potential for a transmitter substance has been defined as the transmembrane potential at which the synaptic current resulting from the transmitter action will be zero (Llinus, Hubbard & Quastel 1969). The transmitter equilibrium potential has been used by Werman et al. (1968) to characterise a particular transmitter substance. It is argued that each transmitter substance results in slightly different post-synaptic ionic chnages. The equilibrium potential will provide, it is suggested, a means of discriminating between different transmitter substances and also of course between the post-synaptic effects of exogenous and endogenously released agents.

Interneuronal activity and 5-HT

The excitatory action of 5-HT on motoneurone excitability demonstrated by the effect of conditioning stimulation, the effect of precursors and antagonists of 5-HT and the local application of drugs, contrast markedly with the response of spinal polysynaptic pathways to 5-HT.

Results presented here suggest that the release of 5-HT leads to a reduction in excitability of some spinal interneurones. Stimulation of raphe nuclei which led to an increase in motoneurone excitability (indicated by an increased MSR amplitude) concomitantly reduced PSR amplitude. The observations of the blocking of this inhibitory effect of conditioning stimulation on PSR activity by 5-HT antagonists,

suggests that the inhibitory effect is mediated via a serotonergic mechanism.

Further evidence of the involvement of 5-HT in the control of interneuronal activity is provided by the precursor study. Fig.33 illustrates the effect of L-tryptophan (i.e. 5-HT) on both the unconditioned and conditioned reflex at various dorsal root stimulus intensities. The smaller amplitude reflex reflects activity in a limited number of motoneurones. Only at this lower level of activity did L-tryptophan depress MSR amplitude. At the higher dorsal root stimulating intensities, the precursor markedly increased both the unconditioned and conditioned reflex amplitudes.

It may be argued that at the lower stimulus intensities when few motoneuones are active, the tonic influence of interneuronal pathways is then more apparent than when all available motoneurones are active.

At lower stimulus intensities few motoneurones are stimulated sufficiently strongly to produce an action potential, however many will be 'subliminally' excited, i.e. excited to a level insufficient to trigger an action potential. These neurones will then be extremely sensitive to interneuronal influences, whereas at the higher dorsal root stimulation intensities, the motoneurone pool is less able to respond to the possibly relatively small interneuronal influences, since it is responding in a supramaximal fashion. The reduction in the MSR corresponding to lower stimulus intensities could then be explained

by the reduced excitatory or active inhibitory influence of spinal polysynaptic pathways. This change in interneuronal activity associated with the administration of L-tryptophan, is presumably due to the formation of 5-HT.

It is difficult to assess the exact nature or mechanism of the effect of raphe-spinal activity on interneuronal activity. Although it is possible to explain these inhibitory effects by postulating a direct inhibitory role for 5-HT at spinal interneurones, the excitatory effects of 5-HT on motoneurones provide little support for the postulate. More acceptable is the simpler explanation that 5-HT released from raphe-spinal neurones excites inhibitory spinal interneurones.

The results presented and discussed here differ from some previous reports. Although there is general agreement concerning the effect of precursors of 5-HT on the MSR, there are considerable differences in the reported effects of precursors on PSR activity.

Although two groups of workers report that 5-HTP reduces the PSR amplitude (Anderson & Shibuya 1966; Banna & anderson 1968), a third publication suggests that 5-HTP increases PSR amplitude (Marley & Vane 1967). The possibility that 5-HTP may activate adrenergic neurones has already been mentioned, it seems likely that such effects may account for some of the above inconsistencies in the results.

The conversion of L-tryptophan to 5-HT involves the additional step of hydroxylation, this probably ensures that 5-HT is only formed at normal physiological sites of

synthesis, where its release would only affect tryptaminergic receptors. It is interesting to note that there is general agreement in the literature that L-tryptophan increases PSR activity. This effect conflicts with the results presented here, however the totally different experimental procedures may account for these differences. (For example, there is no published report concerning the effect of raphe-spinal stimulation on polysynaptic activity).

In conclusion, the present study has shown that 1) stimulation of the nucleus raphe medianus increases motoneurone excitability and decreases at least some interneuronal activity and 2) the modification of motoneurone excitability involves the action of 5-hydroxytryptamine close to the motoneurone. Although it is possible that the excitatory effects are mediated via interneurones close to the motoneurone in the ventral horn, the existence of 5-HT terminals on motoneurones strongly supports the hypothesis that 5-HT has its effect directly on the motoneurone. The study does not provide conclusive evidence that 5-HT is acting as a neurotransmitter at the lumbar spinal motoneurones, however, the results strongly support existing evidence for such a role.

Future work designed to clarify the reported differences in the effects of 5-HT on PSR and MSR activity, may involve the effect of locally applied 5-HT on identified spinal interneurones. Early studies in barbiturate anaesthetised cats suggested that 5-HT had no effect on spinal neurones (Curtis & Davis 1962). In a series of

unanaesthetised spinal cats Engberg & Ryall (1966) reported that 5-HT depressed activity in about 30% of interneurones artificially excited with DL-homocysteic acid. The remaining interneurones were unaffected. Clearly further studies involving the local application of drugs are required.

Although there seems little doubt that 5-HT effects an increase in excitability of motoneurones, little is known of the site of this modification. The fluorescent histochemistry technique provides no information concerning the detailed relationship between the 5-HT containing terminals and spinal neurones. Employing the combined techniques of electron microscopy and autoradiography, it should be possible to localise the position of the serotonergic synapse much more exactly. In addition to its intrinsic interest, the identification of 5-HT synapses on dendrites and cell bodies will provide information concerning the function of the pathway. If, for example, 5-HT synapses were found predominantly in association with dendrites, it would suggest that the raphe-spinal pathway would mediate a tonic type control of excitability (Fadiga & Brookhart 1960; Rall et al.1967). The anatomy of the descending pathway (i.e. the fine unmyelinated nature of the serotonergic fibres) together with the apparent weak pharmacological action of 5-HT (as demonstrated by iontophoretic studies) supports the theory that activity in the 5-HT system is of the tonic type.

A more immediate and profound significance of the present study lies in the identification of a pathway whose activity is mediated by 5-HT. The comparative

simplicity and accessability of the pathway means that for the first time the effects of psychoactive drugs can be specifically investigated on activity in a tryptaminergic pathway.

Although reports have recently been published concerning the interaction of tricyclic antidepressants and 5-HT precursors on spinal MSR transmission (Clineschmidt, Pierce & Sjoerdsma 1971), the technique suffers from the criticism that the modification of MSR activity is the final expression of the interaction between the antidepressant agent and other transmitter substances in the spinal cord. By examining the effect of psychoactive drugs on the conditioned MSR, it will be possible to determine the interaction between the drug and transmission in a 5-HT-containing pathway.

R E F E R E N C E S

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